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(54) Title: PARATHYROID HORMONE ANALOGUES MODIFIED AT POSITIONS 3, 6 or 9

(57) Abstract

The present invention relates polypeptide analogs which have agonist or antagonist or tissue selection properties relative to parathyroid hormone (PTH), parathyroid hormone-like protein (PLP) or parathyroid-related protein (PTHrP). The serine amino acid at position 3, the glutamine amino acid at position 6, the histidine amino acid at position 9 or combinations thereof are substituted by other natural or synthetic amino acids. Preferably, a human PTH fragment of about 34 amino acids is sufficient for pharmacological activity. These polypeptides are useful as agonists or antagonists in the treatment of a human being for disease conditions of cancer, osteoporosis, hypercalcemia, or hyperparathyroid disease conditions. The invention also concerns a method of performing certain assays using the modified peptides, and based on the results of the assays falling within preset limits, selecting those modified peptides which shall be useful in the treatment of disease conditions.

PARATHYROID HORMONE ANALOGUES MODIFIED
AT POSITIONS 3, 6 OR 9

BACKGROUND OF THE INVENTION

Related Applications

The present application is a continuation-in-part of Serial Number 553,760 filed July 13, 1990, which is incorporated herein by reference.

Origin of the Invention

5   The research disclosed herein was supported in part by the U.S. National Institutes of Health Grants Nos: GM 39900, CA 34738, and AM 35323, and by the Research Service of the Veterans Administration. The U.S. Government has certain rights in this invention.

10   Field of the Invention

The present invention relates to polypeptide analogs which have agonist or antagonist properties relative to parathyroid hormone, (PTH), parathyroid hormone-like protein (PLP) or parathyroid-related protein (PTHrP). The
15   serine amino acid at position 3, the glutamine amino acid at position 6, or the histidine amino acid at position 9, or combinations thereof, are substituted by other natural or synthetic amino acids. Preferably, a human PTH fragment of about 34 amino acids is sufficient for useful
20   pharmacological activity. These polypeptides are useful in the treatment of a human being for conditions of cancer, osteoporosis, hypercalcemia, or hyperparathyroid disease.

Description of Related Art

The search for potent PTH agonists and/or antagonists
25   has been intensive. The availability of potent and specific antagonists would provide a powerful research tool for the study of the mechanism of action and physiological and/or pathological role for PTH. Some research efforts have resulted in in vitro PTH antagonists. However, during
30   in vivo evaluation of these polypeptides, they often did not have any clear antagonist properties.

For a number of polypeptide hormones, discrete, localized structural modifications are sufficient to

convert a receptor agonist to a competitive receptor antagonist. Underlying this observation is the idea that the distinct functions of receptor-binding of hormone and initiation of biologic action, are signalled by distinct

5 structural domains within the polypeptide hormone sequence. Parathyroid hormone (PTH) is a well-studied example of such a polypeptide hormone. PTH(1-34) is a full agonist of the native 84 amino-acid hormone with respect to adenylate cyclase activation in canine renal membranes (See Ref. 1

10 below. The letters used are the conventional ones to describe an amino acid sequence). Amino-terminal truncation results in polypeptides that are competitive antagonists of PTH-stimulated adenylate cyclase. Thus [Tyr^{34}]bPTH(7-34)amide retains moderate affinity for renal

15 PTH receptors, but does not have any agonist activity. Specific weak receptor binding activity is retained in a fragment as small as PTH(25-34) (Ref. 2). On the other hand, carboxyl-terminal truncations of PTH(1-34) produce agonists with progressively lower affinities. PTH(1-25) is

20 reported to be essentially inactive (Ref. 3-5). The "receptor-binding domain" of PTH is believed to include amino acid residues 25-34 and the "activation domain" includes amino acid residues 1-6.

A recently identifed tumor-derived protein bears

25 limited sequence identity with PTH (Ref. 6-8) and activates PTH-responsive adenylate cyclase with a potency comparable to that of PTH (Ref. 9-11). This 139-141 amino-acid PTH-related protein (PTHrP), and synthetic amino-terminal fragments derived therefrom, display high affinity for

30 renal and skeletal PTH receptors (Ref. 12-15). They also reproduce the major biologic actions of PTH _in vivo_ and _in vitro_ (Ref. 12,15-18). PTH-related protein is found in a variety of human and animal tumors, and evidence suggests that the protein plays a pathogenetic role in the

35 hypercalcemia that frequently accompanies malignant disease (Ref. 19,20). The amino acid sequence similarities between mammmalian PTHs and PTHrP are largely limited to the amino-

terminal 1-13 residues, of which 8 are identical, See
Figure 1. Although PTHrP displays a high affinity for PTH
receptors, only 1 of 10 amino acids in the 25-34 receptor-
binding domain of PTH is common to PTHrP. Furthermore, a

5 polypeptide analogue of PTHrP(14-38) is reported to bind
(with low affinity) to PTH receptors in ROS 17/2.8 rat
osteosarcoma cells (Ref. 21). Thus, conformational
similarity rather than a strict conservation of sequence
may underlie this interaction of PTH and PTHrP with a

10 common receptor.

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5   All references, articles, patents, standards, etc. cited at any point in this application are incorporated herein by reference in their entirety.

Reference numbers in parenthesis in the text are to the numbers of the references found in this section.

10   At the present time a number of PTH analogs and studies have been reported for replacement of amino acids primarily the 10-34 region of the polypeptide chain. However, few hPTH or bPTH, etc. analogs have been reported where the amino acids are substituted in the 1-9 amino acid

15 positions. The present invention provides a method to select and to produce novel hPTH or bPTH, etc. analogs where the amino acids at positions 3 and/or 6 and/or 9 have been replaced using the natural or synthetically made unnatural (unusual) amino acids. These analogues replaced

20 at the 3, 6, or 9 positions have surface side chains which are useful to modulate receptor binding and activity. These analogues are useful as agonists or antagonists in the treatment of a number of disease conditions, particularly osteoporosis.

25   SUMMARY OF THE INVENTION

In one aspect, the present invention concerns a pharmaceutical composition comprising a compound of the formula:

H_2N-(Ser or Ala)^1-Val-B^3-Glu-Ile-J^6-(Leu or Phe)^7-Met-X^9-

30 Asn^{10}-Leu-Gly-Lys-His-Leu-(Asn or Ser)^{16}-Ser-(Met or Leu)^{18}-Glu-Arg^{20}-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp^{30}-Val-His-Asn-Phe^{34}-Z (Structure I), or

the pharmaceutically acceptable salts thereof, wherein:

35   the amino acid B at position 3 is independently selected from L-serine or those other natural or synthetic D or L amino acids having a spatial volume comparable to or

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greater than serine, with the proviso that B is not glycine,

the amino acid R at position 6 is independently selected from L-glutamine or from other natural or synthetic D or L amino acids or mixtures of the D and L amino acids,

the amino acid X at position 9 is independently selected from L-histidine or other natural or synthetic D or L-amino acids, with the proviso that when group B is L-serine and group J is L-glutamine, Group X is not histidine, when group B is L-serine and group X is histidine, J is not glutamine, and when group J is glutamine and group X is histidine, group B is not L-serine, and

Z is independently selected from -COOH, -COO-+M wherein M+ is selected from pharmacologically compatible cations, -(C=O)NH$_2$, or the sequence of amino acids of human parathyroid hormone, or of human parathyroid hormone-related protein.

In a preferred embodiment the amino acid at position 1 is serine, the amino acid at position 7 is leucine, the amino acid at position 16 is Asn, and the amino acid at position 18 is methionine, i.e. a modified analog of human PTH.

In a preferred embodiment, the amino acid at position 1 is alanine, the amino acid at position 7 is phenylalanine, the amino acid at position 16 is serine, and the amino acid at position 18 is methionine, i.e. a modified analog of bovine PTH.

In other preferred embodiments, Z, B or J are independently selected as follows:

Z is -COOH or -COO-M+ or -(C=O)NH$_2$;

B is a synthetic amino acid;

B is a naturally occurring amino acid;

J is a synthetic amino acid;

J is a naturally occurring amino acid;

B is L-serine and J is selected from Leu, Phe, Ala,

Glu, Ser or Phe; or

J is L-glutamine and B is independently selected from Ala, Phe, Gln, Glu, Lys, His, Leu, or Tyr.

In another aspect, the invention also relates to a compound of wherein J is independently selected from L-Serine, Ala, Phe, Gln, Glu, Lys, His or Tyr.

In yet another aspect, J is independently selected from L-glutamine, Leu, Phe, Ala, Glu, Ser or Phe.

In another aspect, the present invention also relates to a pharmaceutical composition comprising a compound of structure I or a pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable excipient.

In another aspect, the present invention relates to pharmaceutical compositions comprising a compound of the structure for hPTH(1-34) wherein Z is selected -COOH or COO-+M or -(C=O)NH$_2$ (preferably the amide), in admixture, with a pharmaceutically acceptable excipient.

In another aspect, the invention also relates to a method of treatment of a mammal in need of therapeutic treatment, which method comprises administration of a therapeutically effective amount of the peptide analog of a compound of Structure I or a pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable excipient.

In another aspect, the invention also relates to administration of a therapeutically effective amount of a compound of structure I by oral, parenteral, subcutaneous, intramuscular, intravenous, vaginal, rectal, buccal, sublingual or intranasal means.

In another aspect, the invention also relates to a method of therapeutic treatment, wherein the compound of structure I is used to treat cancer, osteoporosis, hypercalcemia or hyperparathyroid conditions in a human being.

In another aspect, the present invention relates to a method for selecting polypeptide sequences modified at the 3, 6, 9 position or combinations thereof of a PTH or PTH(1-

34) useful in pharmaceutical compositions, which method comprises:

(a) preparing an amino acid sequence of hPTH, bPTH, pPTH, hPTHrP, bPTHrP, hPTH(1-34), bPTH(1-34) or pPTH(1-34) wherein the amino acid at the 3, 6, 9 position or combinations thereof, are replaced by different D- or L- natural amino acids or unnatural amino acids;

(b) performing an assay using specific soft tissues, membranes, or cells to evaluate receptor binding and activity;

(c) performing an assay using specific bone cells to evaluate receptor binding and activity;

(d) (i) independently selecting those peptide amino acid analogs for further evaluation, having a high binding and high activity in the specific soft tissues, membranes or cells and a high specific bone cell binding and high activity, as agonists for medical treatment for disease conditions of the soft tissues, membranes, cells or bone, or alternatively;

(ii) independently selecting those peptide amino acid analogs for further evaluation, having a high binding and high activity as agonists in the specific soft tissues, membranes, or cells and a low specific bone cell binding and low activity as agonists for disease conditions of the soft tissues, membranes, or cells, or alternatively;

(iii) independently selecting those peptides for further evaluation as agonists having a low binding and low activity in specific tissues, membranes or cells, and high specific bone cell binding and high activity for disease conditions of the bone, or alternatively;

(iv) independently selecting high binding and low activity in tissues, membranes or cells and in bone cells for use as antagonists in the medical treatment of hormonal disorders and cancers; and

(e) performing subsequent different assays or toxicity determinations on the amino acid analogs pursuant to identifying a useful pharmaceutical.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a representation of aligned sequences of PTH(1-34) from five species of mammals and human and chicken PTHrP(1-34). Positions of sequence identity are 5 highlighted with solid lines.

Figure 1A is representation of the 1-84 amino acids of human PTH, bovine PTH and porcine PTH.

Figure 2 is a representation of secondary structural features of the 1-34 peptides of bPTH(1-34) and human 10 PTHrP(1-34) as predicted by statistical and pattern-based theoretical approaches. The arrows indicate predicted alpha-helical structure, and the wavy line represents a possible beta-turn.

Figure 3 is the circular dichroism (CD) spectra of 15 peptides in the presence and absence of 45% trifluoroethanol. Nadirs at 208 nm and 222 nm are characteristic of a-helical structure. The peptides analyzed are: Figure 3A, bPTH(1-34); Figure 3B [Tyr^{34}]bPTH(7-34)amide; and Figure 3C, hPTHrP(1-34)amide.

20 Figure 4 is a representation of the effect of trifluoroethanol on the alpha-helical content of bPTH(1-34) (o), [Tyr^{34}]bPTH(7-34)amide (Δ), and hPLP(1-34)amide (•). The alpha-helical content was determined by deconvolution of circular dichroism spectra as described by Taylor and 25 Kaiser (Ref. 29).

Figure 5 is a schematic representation of the paired helix model for bPTH(1-34) and hPTHrP(1-34). Positions of relative sequence conservation are shown. The hydrophobic core, binding region, and trigger region are designated.

30 Figure 6 shows graphs of the biologic activity in canine renal plasma membranes of analogues of PTH(1-34) containing substitutions at positions 3,6, and 9.

Figure 6A shows adenylate cyclase (AC) activation produced by bPTH(1-34) (o), and [Glu^3]bPTH(1-34) (■), 35 [His^3]bPTH(1-34) (∇), [Lys^3]bPTH(1-34) (▼), and [Gln^3]bPTH (1-34) (◊).

Figure 6B shows adenylate cyclase (AC) activation

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produced by bPTH(1-34) (O), [Ala³]bPTH(1-34) (●),
[Phe³]bPTH(1-34) (△), [Leu³]bPTH(1-34) (▲), and [Tyr³]bPTH(1-
34) (□).

Figure 6C shows adenylate cyclase activation produced
5   by bPTH(1-34) (O), [Ala⁶]bPTH(1-34) (●), [Phe⁶]bPTH(1-34)
(△) [Leu⁶]bPTH(1-34) (▲), [Ser⁶]bPTH(1-34) (□), [Glu⁶]bPTH(1-
34) (■), and [Phe⁶]bPTH(1-34) (▽).

Figure 6D shows competitive binding to PTH receptors
by bPTH(1-34) (O), [Ala³]bPTH(1-34) (●), [Phe³]bPTH(1-34)
10   (△), [Leu³]bPTH(1-34) (▲), and [Tyr³]bPTH(1-34) (□).

Figure 6E shows competitive binding to PTH receptors
by bPTH(1-34) (O), [Glu³]bPTH(1-34) (■), [His³]bPTH(1-34)
(▽), [Lys³]bPTH(1-34) (▼), and [Gln³]bPTH(1-34) (◊).

Figure 6F shows competitive binding to PTH receptors
15   by bPTH(1-34) (O), [Ala⁶]bPTH(1-34) (●), [Phe⁶]bPTH(1-34)
(△), [Leu⁶]bPTH(1-34) (▲), [Ser⁶]bPTH(1-34) (□), and
[Glu⁶]bPTH(1-34) (■).

Figure 7 shows graphs of the biologic activity in UMR
106-H5 cells of analogues of PTH(1-34) containing
20   substitutions at positions 3, 6, and 9.

Figure 7A shows adenylate cyclase (AC) activations
produced by bPTH(1-34) (O), [Ala³]bPTH(1-34) (●),
[Phe³]bPTH(1-34) (△), [Leu³]bPTH(1-34) (▲), and [Tyr³]bPTH(1-
34) (□).

25   Figure 7B shows adenylate cyclase (AC) activation
produced by bPTH(1-34) (O), [His³]bPTH(1-34) (▽),
[Lys³]bPTH(1-34) (▼), and [Gln³]bPTH(1-34) (◊).

Figure 7C shows adenylate cyclase (AC) activation
produced by bPTH(1-34) (O), [Ala⁶]bPTH(1-34) (●),
30   [Phe⁶]bPTH(1-34) (△), [Leu⁶]bPTH(1-34) (▲), [Ser⁶]bPTH(1-34)
(□), and [Glu⁶]bPTH(1-34) (■).

Figure 7D shows competitive binding to PTH receptors
by bPTH(1-34) (O), [Ala³]bPTH(1-34) (●) [Phe³]bPTH(1-34)
(△), [Leu³]bPTH(1-34) (▲), and [Tyr³]bPTH(1-34) (□).
35   Figure 7E shows competitive binding to PTH receptors
by bPTH(1-34) (O), [Glu³]bPTH(1-34) (■), [His³]bPTH(1-34)
(▽), [Lys³]bPTH(1-34) (▼), and [Gln³]bPTH(1-34) (◊).

Figure 7F shows competitive binding to PTH receptors by bPTH(1-34) (O), [Ala⁶]bPTH(1-34) (•), [Phe⁶]bPTH(1-34) (Δ), [Leu⁶]bPTH(1-34) (▲), [Ser⁶]bPTH(1-34) (□), [Glu⁶]bPTH(1-34) (■), and [Phe⁹]humanPTH(1-34) (▽).

5   Figure 8 shows inhibition of bPTH(1-34)-stimulated adenylate cyclase activity by the partial agonist [Phe⁶]bPTH(1-34).

Figure 8A shows adenylate cyclase (AC) activation in canine renal plasma membranes produced by bPTH(1-34) (O),

10 [Phe⁶]bPTH(1-34) (•), and bPTH(1-34) (5nM) in the presence of varying concentrations of [Phe⁶]bPTH(1-34) (Δ).

Figure 8B shows adenylate cyclase (AC) activation in UMR 106-H5 cells produced by [Phe⁶]bPTH(1-34) (O) and by 0.2 nM bPTH(1-34) in the presence of varying concentrations of

15 [Phe⁶]bPTH(1-34) (•).

DETAILED DESCRIPTION OF THE INVENTION
AND PREFERRED EMBODIMENTS

Definitions

Throughout this application the name of an amino acid

20 may be followed by a superscript number. This designation (e.g. Tyr³⁴) refers to the amino acid at position 34 of PTH be tyrosine.

"B³" refers to the amino acid B at position 3 of PHT, etc.

25   "J⁶" refers to the amino acid J at position 6 of PHT, etc.

"X⁹" refers to the amino acid X at position 9 of PHT, etc.

The references to specific journal(s) are ususally

30 found in parenthesis (Ref.) at the end of a sentence.

"bPHT" refers to the bovine sequence of PTH having a specific sequence of 1-84 amino acids.

"bPHT(1-34)" refers to a shortened bovine PHT sequence, the sequence of active 1-34 amino acids.

35   "cPTH" refers to the 1-84 chicken sequence for PTH.

"hPTH" refers to the 1-84 human sequence for PTH.

"hPTH(1-34)" refers to the shortened amino acid sequence for human PTH.

"hPTHrP" refers to the natural human parathyroid hormone <u>related</u> protein of 139-141 amino acids. bPTHrP is derived from bovine cells. hPTHrP is derived from human cells. pPTHrP is derived from porcine cells.

5   "hPTHrP(1-34)" refers to the active 1-34 amino acids of natural human parathyroid hormone <u>related</u> protein.

"pPTH" refers to the 1-84 porcine anmino acid sequence for PTH.

"pPTH(1-34)" refers to the active 1-34 normal porcine
10 amino acid sequence for pPTH.

As set forth above and for convenience in describing this invention, the conventional abbreviations for the various common amino acids are used as generally accepted in the peptide art as recommended by the IUPAC-IUB
15 Commission on Biochemical Nomenclature, <u>Biochemistry</u>, Vol. 11, 1726 (1972) and represent L-amino acids with the exception of the achiral amino acid glycine. All peptide sequences mentioned herein are written according to the generally accepted convention whereby the N-terminal amino
20 acid is on the left and the C-terminal amino acid is on the right. In some polypeptides of the present invention, the terminal -COOH group is converted to the amide group $-C(=O)NH_2$. The polypeptides are identified as the '-amide'. For example, 'Gly-amide' would designate the
25 terminal group, $-CH_2-C(=O)-NH_2$.

"Natural amino acids" refer to those well known in the art. They are listed and standard abbreviations are provided in the U.S.P.T.O. publication, <u>Trademark Official Gazette</u>, published May 15, 1990, p. 33 at 46. These amino
30 acids and abbreviations are specifically incorporated herein by reference.

The natural amino acids are shown below:

	A	Ala	alanine
	D	Asp	aspartic acid
35	E	Glu	glutamic acid
	F	Phe	phenylalanine
	G	Gly	glycine

15

H	His	histidine
I	Ile	isoleucine
K	Lys	lysine
L	Leu	leucine
M	Met	methionine
N	Asn	asparagine
Q	Gln	glutamine
R	Arg	arginine
S	Ser	serine
T	Thr	threonine
V	Val	valine
W	Trp	tryptophane
Y	Tyr	tyrosine

Modified (unusual, modified or substituted) amino acids are found in the above cited _Trademark Official Gazette_ at p. 47 and 48. Unusual or modified amino acids include but are not limited to these which are specifically incorporated herein by reference:

Aad	2-Aminoadipic acid
bAad	3-Aminoadipic acid
bAla	beta-Alanine, beta-Aminopropionic acid
Abu	2-Aminobutyric acid
4Abu	4-Aminobutyric acid, piperidinic acid
Acp	6-Aminocaproic acid
Ahe	2-Aminoheptanoic acid
Aib	2-Aminoisobutyric acid
bAid	3-Aminoisobutyric acid
Apm	2-Aminopimelic acid
Dbu	2,4-Diaminobutyric acid
Dpm	2,2'-Diaminopimelic acid
Dpr	2,3-Diaminopropionic acid
EtGly	N-Ethylglycine
EtAsn	N-Ethylasparagine
Hyl	Hydroxylysine
aHyl	allo-Hydroxylysine
3Hyp	3-Hydroxyproline
4Hyp	4-Hydroxyproline

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alle	allo-Isoleucine
MeGly	N-Methylglycine, sarcosine
MeIle	N-Methylisoleucine
MeLys	N-Methylvaline
Nva	Norvaline
Nle	Norleucine
Orn	Ornithine
Cit	Citrulline
	3, 4 or 5-Fluorohistidine

Further, unusual or modified "amino acids" include those substituted amino acids which are further substituted on the molecule with another group, such as alkyl or hydroxyl. Typical substituted amino acids include, for example, 4-hydroxy-L-proline, sarcosine ("Sar" also known as N-methylglycine), D-3-(2-naphthylalanine) "D-Nal", N^5-(aminocarboxyl)-ornithine "Cit", pyro-glutamic acid, ornithine ; pmp (1-B-mercapto-beta,beta-pentamethylene propionic acid; Tyr (Et), tyrosine ethylated at the 4-hydroxyl position.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted phenyl" means that the phenyl may or may not be substituted and that the description includes both unsubstituted phenyl and phenyl wherein there is substitution; "optionally followed by converting the free base to the acid addition salt" means that said conversion may or may not be carried out in order for the process described to fall within the invention, and the invention includes those processes wherein the free base is converted to the acid addition salt and those processes in which it is not.

As used herein, the term "pharmaceutically acceptable salts" refers to salts that retain the desired biological activity of the parent compound and do not impart any undesired toxicological effects. Examples of such salts

are (a) acid addition salts formed with inorganic acids,
for example hydrochloric acid, hydrobromic acid, sulfuric
acid, phosphoric acid, nitric acid and the like; and salts
formed with organic acids such as, for example, acetic
5   acid, oxalic acid, tartaric acid, succinic acid, maleic
acid, fumaric acid, gluconic acid, tannic acid, pamoic
acid, alginic acid, polyglutamic acid, naphthalenesulfonic
acids, naphthalenedisulfonic acids, polygalacturonic acid;
(b) salts with metal (M) cations such as sodium, potassium,
10   zinc, calcium, bismuth, barium, magnesium, aluminum,
copper, cobalt, nickel, cadmium, and the like; or with an
organic cation formed from N,N'-dibenzylethylenediamine or
ethylenediamine; or (c) combinations, of (a) and (b), e.g.
a zinc tannate salt and the like.

15   Description of the Invention

     The present invention combines available or derived
structure-activity data with predictive and/or experimental
determinations regarding the molecular structure of PTH, or
PTHrP to identify key amino acid residues in the 1-34 (or
20   larger) amino acid sequences expected to play critical
roles in high affinity receptor binding and activation.
The analogs containing amino acid substitutions at the 3,
6, or 9 positions or combinations thereof are synthesized
and evaluated for the influence of side-chain polarity,
25   charge and size or biological activity.   The in vitro
bioassay is described for PTH receptor binding and
adenylate cyclase activity in canine renal membrane and in
rat bone and human bone cells.   The results of these in
vitro assays are predictive of in vivo biological activity.
30   Two semi-empirical approaches were used to predict the
secondary structural features of mammalian PTH(1-34) and
PTHrP(1-34) - statistical (Ref. 22,23) and pattern-based
(24,25) (Figure 2).   For PTH(1-34) two helical segments
comprising 68% (23/24) to 82% (28/34) of the peptide are
35   predicted, as previously suggested (Ref. 26,27).   The
putative COOH-region helix contains residues thought to
play a direct role in hormone binding to its receptor,

whereas the NH_2-region helix would include either part of or all of the 1-6 peptide segment required for activation for the receptor (induction of coupling to the stimulatory GTP-binding component of adenylate cyclase, G_s. Similarly, two

5   helical segments are predicted for PTHrP(1-34). These would comprise 74% (23/34) to 94% (32/34) of the peptide. Chou-Fasman (Ref. 22) calculations suggest a beta-turn in positions 10-13 of PTH(1-34), and strongly predict a beta-turn in the corresponding region of PTHrP.

10   Circular dichroism (CD) spectra for bPTH(1-34), [Tyr[34]]bPTH(7-34)amide, and hPTHrP(1-34)amide were obtained in an aqueous buffer in the presence and absence of trifluoroethanol (TFE). In the absence of TFE, bPTH(1-34) yielded a spectrum with a nadir at 208 nm and a broad

15   shoulder in the region of 222 nm (Figure 3). In the presence of 45% TFE, the nadir at 208 nm deepened, and a second minimum appeared at 222 nm. These results suggest the presence of alpha-helical structure for bPTH(1-34) that is enhanced in the presence of TFE. The ellipticity became

20   positive at wavelengths less than 200 nm, indicating the presence of a small amount of residual beta-structure. Surprisingly, this feature was also enhanced by TFE. The spectra qualities exhibited by hPTHrP(1-34)amide and [Tyr[34]]amide (7-34) were qualitatively similar.

25   The relationship between alpha-helical content (estimated by deconvolution of CD spectra according to Taylor and Kaiser, Ref. 29) and solvent amphiphilicity for these peptides is presented in Figure 4. The spectra were consistent with progressively increasing alpha-helical

30   structure with increasing concentrations of TFE. In the presence of 45% TFE, bPTH(1-34) and hPTHrP(1-34) amide were calculated to display, respectively, 25 ± 3 and 24 ± 3 residues contained within alpha-helical domains. These results are predicted by the Chou-Fasman analysis described above. The

35   CD spectrum of the PTH(7-34) analogue was also consistent with the Chou-Fasman analysis. PTH(7-34) contained 7 to 8 fewer alpha-helical residues than either bPTH(1-34) or

hPTHrP(1-34)amide, providing direct evidence to suggest
that the amino-terminal 1-6 domain of PTH assumes or
induces alpha-helical structure in the presence of TFE.
This result was confirmed in a second experiment in which

5   [Tyr^{34}]bPTH(1-34)amide and [Tyr^{34}]bPTH(7-34)amide were
calculated to display, respectively 24 and 18 alpha-helical
residues in the presence of 30% TFE.

In the absence of TFE, bPTH(1-34), hPTHrP(1-34)amide,
and [Tyr^{34}]bPTH(7-34)amide are calculated to have 7-10

10 alpha-helical residues, consistent with a second alpha-
helical domain that persists under strictly polar solvent
conditions. This latter domain is predicted to lie between
residues 17-34 of PTH and PTHrP. These results are similar
to previous estimates of less than 12 alpha-helical

15 residues for bPTH(1-34) and hPTH(1-34) (37-39), and 9
alpha-helical resides for bPTH(7-34) (Ref. 37) under
aqueous conditions in the absence of amphiphiles. The
effect of TFE on the secondary structure of the PTH(7-34)
analogue may reflect extension of the putative COOH-region

20 helical domain under conditions of increasing solvent
amphiphilicity.

The results indicate that, under appropriate solvent
conditions, both bPTH(1-34) and hPTHrP(1-34)amide have
extensive secondary structure that is largely alpha-

25 helical. Based on these results, a three-dimensional model
of the 1-34 sequences of PTH and PTHrP was constructed by
packing the amino-and carboxyl-terminal α-helices together
(Figure 5). A hydrophobic contact surface on the face of
each helix was located using the method of Richmond and

30 Richards (Ref. 40). A helical assembly was constructed
following the approach of Cohen et al. (Ref. 41). To
maintain the connectivity of the chain, the interhelical
packing angle was necessarily +20 or -20° (+160° or -160°).

In the context of this model, conserved substitutions

35 at positions 4, 7, and 8 as well as 20, 23, 24, 28, and 31
involve residues buried in the hydrophobic core.
Presumably these r sidues contribute to the stabilization

of the folded structure. By contrast, conserved solvent-
exposed residues at positions 3, 6, 9, and 12 are likely to
participate in critical interactions at the ligand-receptor
interface. Twenty-three analogues of bPTH(1-34) monosub-
5 stituted at positions 3 or 6 or 9 or disubstituted at
positions 3 and 6 were synthesized generally following the
procedures for peptide amino acid synthesis and
specifically as found in the Experimental section and
tested for their inactivity in binding to PTH receptors and
10 activating adenylate cyclase in canine renal plasma
membranes and UMR 106-H5 rat osteosarcoma cells (Figures 6
and 7, and Tables 1 and 1A). Effects of these
substitutions on receptor binding were similar in the
kidney and bone systems. At position 3, a diverse array of
15 amino acids including Ala, Leu, Gln, and His were well
tolerated (greater than 30% retention of binding potency)
whereas Lys, Phe, and Tyr were less well tolerated (2-15%
retention of binding potency). [Glu^3]bPTH(1-34) retained
less than 0.1% receptor binding activity. Substitutions at
20 position 6 resulted in analogues with moderately reduced
binding activity, with the exception of [Phe^6]bPTH(1-34)
which retained only 1% (renal membranes) 4% (osteosarcoma
cells) of the activity of bPTH(1-34).

In general, reductions in binding affinity were
25 paralleled by a comparable increase in the concentration of
peptide required for stimulation of adenylate cyclase in
UMR 106-H5 cells. Exceptions were [Lys^3] and [Phe^3]bPTH(1-
34) which exhibited disproportionately lesser and greater
losses in cyclase-stimulating activity, respectively, than
30 expected from their binding affinities. In contrast,
virtually all of the analogues displayed disproportionately
low cyclase-stimulating potencies in renal membranes. A
similar disparity between renal membrane binding affinity
and cyclase-stimulating activity has been noted for
35 PTHrP(1-34) (Ref. 14). Thus, most of the analogues as well
as PTHrP are apparently less efficacious than bPTH(1-34) in
inducing coupling of the PTH receptor to the activation of

21

adenylate cyclase in canine renal plasma membranes.

Four synthesized analogues exhibited partial agonist activity in the adenylate cyclase assay. Three of these analogues (Phe[3], Tyr[3], Phe[6]) involved substitution of
5 hydrophobic residues, whereas the fourth involved a Ser for Gln substitution at position 6. Analogues that exhibited partial agonist behavior did so in both the bone and kidney systems, although to somewhat varying extents. The weak partial agonists were able to inhibit competitively the
10 adenylate cyclase response to the full agonists PTHrP(1-34) and bPTH(1-34) (Figure 8).

Assays were conducted using UMR-160-H5 cells. These assays were performed as described in Cohen et al. (1991), J. Biological Chem., 266(3), 1997 and in the Experimental
15 section, e.g. Example 11.

In UMR-106-H5 cells, several of the position 3 analogues displayed increased potency for adenylate cyclate activation (Leu[3], His[3]) or increased activity in adenylate cyclase (Tyr[3]), compounded with renal adenylate cyclase.
20 This result demonstrates that these analogues are relatively bone specific.

Although the analogues tested displayed a wide range of potencies in the receptor binding adenylate cyclase assays, the substitutions produced little effect on
25 secondary structure as evidenced by CD spectroscopy. The calculated alpha-helical content varied only between 29-36% for these peptides, with exception of [Glu[3]]bPTH(1-34) which displayed 19% alpha-helical content (Table 1). The reduced alpha-helical content of [Glu[3]]bPTH(1-34) was associated
30 with a dramatic loss of biological activity in both assay systems. For the remainder of the analogues, alterations in biological activity appeared to be independent of major changes in secondary structure.

In Table 1 below, the potency and activity of bPTH(1-
35 34) is designated as 100%, and the peptide analogues are described in percent relative to the bPTH(1-34). The renal membrane assay values listed reflect the effect of the

polypeptide analogue with kidney tissue receptors. The UMR-106-H5 assay values reflect the effect of the peptide analogue with rat bone tissue receptors. The Sa05-2 assay values reflect the effect of the peptides for human bone tissue receptors.

Activity is defined as the intrinsic ability to stimulate adenylate cyclase so that a full agonist would have 100% activity. A full antagonist would have 0% activity. A partial agonist would have >0 and <100% activity. An ideal antagonist in either assay would have 100% binding (B) and 0% activity (AC). A superagonist would have greater than 100% (e.g. 200%) binding (B), and 100% activity (AC) in either assay.

A preferred embodiment is a bone specific peptide analogue which would have binding (B) which approaches 0% and 0% activity in soft renal membranes, and approaches or is greater than 100% binding and activity in the UMR 106-H5 assay.

For example, analog [Tyr^3 bPHT (1-34)] in Table 1 shows a preferred pattern having low binding and activity in renal tissue and high bind and activity in the UMR-106-H5 assay.

Table 1A describes the potency and activity of hPTH (1-34) designated as 100% and the peptide analogs are described in percent relative to hPTH (1-34) (human).

TABLE 1 – BOVINE PTH ASSAY RESULTS

	RENAL MEMBRANES Canine			UMR106-H5 CELLS Rat Bone			SAOS2 CELLS Human Bone		% HELIX TFE	
	Potency (B)	(AC)	Activity (AC*)	Potency (B)	(AC)	Activity (AC*)	Potency (AC)	Activity (AC)	(0%)	(30%)
bPTH(1-34)	100	100	100	100	100	100			35	91
ala[3]	79	100	89	38	79	90			30	100
leu3	44	3.3	85	45	52	92			ND	ND
phe[3]	22	1	39	8	2	80			ND	ND
gln[3]	57	35	100	47	100	100			31	95
glu[3]	<0.1	<0.1	>30	0.2	<0.1	>35			19	93
lys[3]	14	1.5	78	3.5	14	95	20	100	29	81
his[3]	55	2.5	100	9	75	100			35	84
tyr[3]	2	1.2	28	0.9	2.5	100			34	99
leu[6]	12	3.5	84	6.3	7	81			33	94
phe[6]	1.3	1.4	26	4.5	1.1	37			33	99
ala[6]	54	4	87	12	22	90	4	80	33	94
glu[6]	72	12	100	48	45	100			36	90
ser[6]	15	1.3	50	33	4.7	35			ND	ND

Potency and maximal activity for PTH receptor binding (B) and adenylate cyclase activation (AC) for 3, 6 and 9-position modified analogues of bPTH(1-34). Modified analogues are Z=amide.

Refer to Table 1A for definition of B, AC and AC*.

Both are shown as % relative to PTH(1-34) amide, which is assigned a value of 100%. The activity of adenylyl cyclase produced by a maximally effective dose of modified peptide is shown relative to that produced by bPTH (1-34). Also shown is the % of alpha-helical structure calculated from circular dichromism (CD) spectra obtained in the presence and absence of 30% trifluoroethanol (TFE), as described in the EXPERIMENTAL section.

TABLE 1(A) – HUMAN PTH ASSAY RESULTS

	RENAL MEMBRANES Canine		UMR106-H5 CELLS Rat Bone			SAOS2 CELLS Human Bone		% HELIX TFE	
	Potency (B)	Activity (AC*)	Potency (B)	(AC)	Activity (AC*)	Potency (AC)	Activity (AC)	(0%)	(30%)
hPTH(1-34)	100	100	100	100	100	100	100	35	91
gln9	7	2	0.8	1.4	100	100	100	37	86
ser9	4	0.4	0.4	0.6	97			32	83
leu9	0.2	1.8	0.6	<0.1	80			40	85
glu9	25	4	6	11	100			39	91
phe9	0.7	1.5	1.8	0.6	84			ND	ND
ala9	0.7	1	0.5	0.1	85			36	83
lys3ala6	0.5	2	<0.1	0.3	72	10	8	33	46
his3ala6	0.2	2	<0.1	0.4	44	14	6	33	47
lys3glu6	89	10	10	10	100	12	100	34	100
his3glu6	8.9	3	0.3	0.5	100	5	100	30	89

Potency and maximal activity for PTH receptor binding (B) and adenylate cyclase activation (AC) for 3, 6 and 9-position modified analogues of bPTH(1-34). Modified analogues are Z=amide.

AC is the concentration of modified peptide half maximal enzyme activation (i.e. the mid-point of the curve transition - can be determined from the enclosed figures.

AC* defines the enzyme activity of large doses of modified peptide relative to PTH reference at 100%.

Both are shown as % relative to PHT(1-34) amide, which is assigned a value of 100%. The activity of adenyl cyclase produced by a maximally effective dose of modified peptide is shown relative to that produced by hPTH(1-34). Also shown is the % of alpha-helical structure calculated from circular dichromism (CD) spectra obtained in the presence and absence of 30% trifluoroethanol (TFE), as described in the EXPERIMENTAL section. ND is not determined.

25

The CD data shown are consistent with our prediction on theoretical grounds that PTH(1-34) and PTHrP(1-34) assume alpha-helical structure in both the amino and carboxyl-terminal domains. Therefore, if the peptides are

5 viewed as an ensemble of structures in equilibrium, containing amino-terminal and carboxyl-terminal alpha-helices, increasing concentrations of TFE presumably drive the equilibrium towards increased helical content. Previous results indicated in a similar increase in the

10 alpha-helical content of hPTH(1-34) in the presence of dimyristoylphosphatidylcholine vesicles (Ref. 42). The nonpolar environment of the specific receptor could also stabilize highly structured forms of PTH and PTHrP.

The proposed model yields a domain structure that

15 includes a hydrophobic core, hydrophilic faces of each helix, and loop between helices. To maximize the burial of hydrophobic surface areas in bPTH and to promote a favorable interaction between the helix dipoles (Ref. 43), a relatively parallel arrangement (interaxial angle - 160°)

20 is expected (see Figure 5). This packing angle is commonly found in helical proteins (Ref. 44,45). An analogous paired helical structure has been demonstrated for 28-residue avian pancreatic polypeptide by X-ray crystallography (Ref. 46).

25 Barden and Kemp (Ref. 28) recently reported the use of 2D-NMR and CD spectroscopy to characterize the three dimensional structure of PTHrP(1-34)amide and its fragments at pH 4.5. They concluded that the peptide assumes a structure consisting of an N-terminal helix (residues 3-9),

30 two reverse turns (residues 10-13 and 16-19), and C-terminal coil (residues 23-34). Most importantly, these investigators detected NOE cross-peaks between distant residues (Ref. 2-31, 8-28, 11-24) confirming the existence of a compact structure. Helical structure was assigned to

35 the amino-terminal region instead of the carboxyl-terminal region based on an analysis of the $^3J_{\alpha CHNH}$ coupling constants and the CD spectra of PTHrP analogues. The region

identified by Barden and Kemp as helical (residues 3-9) has an average $^3J_{\alpha CHNH}$ of 5.4 Hz. Residues 24-30 in the C-terminal region have a similar average $^3J\alpha_{CHNH}$ of 6.1 Hz. However, the CD spectra of PTHrP(1-34), PTHrP(1-25), and

5   PTHrP(7-34) in water were interpreted as showing evidence of an amino-terminal helix only. The CD spectra for bPTH(13-34) and bPTH(19-34) also indicated little if any alpha-helical structure in these peptides under aqueous condition (Ref. 37).

10   Modifications of PTH(1-34) that destabilize either interhelical interactions or helices themselves should result in reduced biological activity. This appears to be the case for analogues of PTH that have been examined. Disruption of the hydrophobic core either by oxidation of

15   Met[8] to SOMet (in bPTH[1-84]) or by a Leu[28] to Lys[28] substitution in [Gln[22], Lys[28], Leu[30]]hPTH(1-34) results in marked (10-50 fold) loss of biological activity (Ref. 49, 50). In contrast, exchanging of or addition of hydrophobic moities as in Met to Nle[8,18], Met to Met-butyl[8,18], Met to Met-

20   butyl[8,18], or NPS-/NAPS-Trp[23] analogues results in little or no loss of bioactivity (Ref. 4, 51-53). Analogues which delete the C-terminal carboxylate (PTH[1-34]amide; [Tyr[34]]PTH[1-34]amide; [D[Tyr[34]]PTH-(1-34amide) have enhanced biological activity (Ref. 54). If residues 23-34 form an

25   alpha-helix, the relative alignment of peptide dipoles creates an effective negative charge at the C-terminum equal to approximately -1/2e (Ref. 43). A C-terminal COO⁻ destabilizes the helix through an unfavorable interaction with the dipole. By contrast, a C-terminal amide

30   stabilizes the helix, perhaps increasing biological potency. Similarly, Lys[13] (within the proposed loop region) stabilizes the dipole of the putative N-terminal helix in PTH an dPTHrP. Indeed, deletion of Lys[13] from PTH(1-34) results in a peptide with markedly reduced bioactivity

35   (Ref. 55). In contrast, oxidation of Met[18] is not expected to destabilize interhelical interactions, and [SOMet[18]]bPTH(1-84) retains nearly 50% of the activity of

the native hormone (Ref. 49).

In the present invention, a combination of spectro-
scopic and predictive methods are used to develop a
structural model for PTH (1-34) (F.E. Cohen et al., _J._
5 _Biol. Chem._ 266:1997, 1991). The peptide is envisaged to
consist of two amphipathic α-helices separated by a loop.
The amino-region α-helix initiates at or near the amino-
terminus and extends to approximately residue 10. The
second α-helix initiates at about residue 18 and extends to
10 the carboxyl-terminus. The helices are suggested to fold
back on one another in an antiparallel fashion, resulting
in formation of a hydrophobic core with externally-facing
polar residues that are expected to be centrally involved
in receptor binding.

15   Three such polar residues are modified: Ser at
position 3; Gln at position 6; and His at position 9.
These residues are conserved in all known members of the
PTH/PTHrP family (Fig. 1), and may thus interact directly
with key receptor determinants. In each case, analogs are
20 synthesized to assess the impact of changes in charge,
hydrophobicity, and side-chain volume.

(i) _Analogs with altered ability to activate_
adenylate cyclase once binding occurs.

In the present invention, several peptides displayed
25 a reduced ability to activate adenylate cyclase at
maximally effective concentrations. The reduced intrinsic
activity of such analogs presumably reflects their failure
to convert the receptor to a fully-functional conformation
subsequent to binding. For the most part, analogs
30 displaying this behavior have hydrophobic residues
substituted for normally polar amino acids at positions 3,
6 and 9. At position 3, substitution of Phe or Tyr for Ser
markedly reduced the intrinsic activity of PTH (1-34). As
discussed below, this effect is specific for the renal
35 assay, and is not evident in bone cells. The reduced
bioactivity is directly related to the volume of the
hydrophobic side-chain introduced at position 3, suggesting

that this residue is sterically constrained within the receptor pocket. A significant negative correlation was obtained between side-chain volume at position 3 (calculated according to Richards (Ref. 57)) and biological
5 activity in each of the assays. For the various assays, the correlation coefficients ranged from -0.68 (p less than 0.05) to - 0.92 (p less than 0.01). Substitution of Phe of Gln at position 6 of PTH (1-34) results in an analog with markedly reduced intrinsic activity in both the renal
10 and bone cell adenylate cyclase assays. Hydrophobic residues are very poorly tolerated at position 9, where substitution of Ala, Leu, or Phe for His produced a loss of intrinsic activity in the renal adenylate cyclase assay. In general, substitution at these positions with polar
15 amino acids produce less of intrinsic biological activity. Exceptions were Ser^6 and Ser^9 PTH(1-34) each of which displays reduced activity.

Analogs that retain receptor-binding activity, but have reduced intrinsic activity are predicted to act as
20 partial PTH antagonists. This is found to be the case with the two partial agonists tested-[Phe^3] and [Phe^6]PTH(1-34). The finding that single substitutions convert PTH(1-34) to a partial antagonist is a novel finding and suggests new approaches to the development of potent PTH receptor
25 antagonists.

(ii) _Effects of substitutions on secondary structure_:

Circular dichroism is used in the art and in this invention to assess the effects of amino acid substitutions on the secondary structure of PTH(1-34). Under aqueous
30 solvent conditions, virtually all of single-substituted analogs display 30-40% α-helical content. The only exception is [^{3}Glu]PTH(1-34) which displays only 19% α-helical content, and shows a marked reduction in biological activity. In 30% trifluorenthanol, solvent conditions
35 similar to those expected in the plasma membrane environment, all of the analogs with single residue substitutions displaying 85% α-helical content. Thus, the

aforementioned loss of intrinsic activity are not attributed to a gross change in the secondary structure of the analogs. As described below, combined substitutions at residues 3 and 6 have a profound effect on receptor-active

5   structure and on biological activity.

(iii)   Peptide Analogs with altered target cell selectivity.

Certain modifications result in disparate losses of activity in the renal vs. bone cell bioassays.

10   Substitutions of the basic residues Lys or His for Ser^3 produces analogs which retained substantial activity in bone cells, but had <3% of the activity of PTH (1-34) in renal membranes. Likewise, $[Glu^6]$ and $[Ala^6]PTH(1-34)$ retain significantly more activity in rat bone cells than

15   in the renal membrane bioassay. The target cell selectivity of $[Ala^6]PTH(1-34)$ appears to be species-dependent since the analog is equipotent in human bone cells and in renal membranes. In contrast, $[Lys^3]PTH(1-34)$ has enhanced bioactivity in both bone cell assays, and may

20   thus discriminate between the PTH receptor-adenylate cyclase system in kidney vs bone.

(iv)   Peptide analogs with double substitutions.

Substitutions are in positions 3 and 6 which individually do not impact markedly upon activity are

25   introduced in pairs to create a series of analogs substituted in both positions. Lys^3ala^6 b-PHT and his^3ala^6 b-PHT show low receptor affinity and low cyclase-stimulating activity in bone cells and in membranes from bone cells and kidney. Since single substitutions of

30   alanine or glutamic acid for glutamine at position 6 or of lysine or histamine for serine at position 3 have minimal effects on intrinsic activity, the result suggests that effective activation of the PTH receptor involves an interaction between positions 3 and 6 in the ligand. This

35   interaction evidently preserves helical structure, as the helical content of analogs in which alanine is substituted for glutamine at positions 6 have reduced helical cont nt.

In contrast, lys^3ala^6 and his^3ala^6 are full agonists in all systems. It appears that with a small side-chain in the 6 position, basic residues at position 3 are markedly destabilizing and detrimental to binding, as are other

5 amino acid substitutions which destablize the helix structure.

The amino acid in position 9 is the natural one histidine or is preferably one which will easily accept a positive charge, and thus modify the helical structure,

10 e.g. tyrosine, tryptophan, phenylalanine, diaminobutyric acid, D-Nal, ornithine, citrulline, 3,4 or 5-fluoro-histidine and the like.

Selection of Modified Polypeptides Useful as Pharmaceuticals.

15   In an additional aspect of the present inveniton, by use of the assays and their results, it is possible to select those modified peptides which are active for further evaluation to produce useful pharmaceuticals.

This result is accomplished by modifying the peptide

20 (e.g. PTH(1-34) at the 3,6 or 9 position or a combination thereof as described herein,

performing an assay using specific soft tissue, membrane or cells, to evaluate receptor binding and activity; and

25   performing an assay using specific bone cells to evaluate solid bone receptor binding and activity.

Based on the results of the assay, four categories of useful pharmaceuticals are determined, i.e.

(d) (i) independently selecting those peptide amino

30 acid analogs for further evaluation, having a high binding and high activity in the specific tissues, membranes or cells and a high specific bone cell binding and high activity, as agonists for medical treatment for disease conditions of the tissues, membranes, cells or bone, or

35 alternatively;

(d) (ii) independently selecting those peptide amino acid analogs for further evaluation, having a low binding

and high activity as specific agonists for medical treatment in the specific soft tissues, membranes, or cells and a high specific bone cell binding and low activity for disease conditions of the tissues, membranes, or cells, or

5 alternatively;

(d)   (iii) independently selecting those peptides for further evaluation as agonists having a low binding and low activity in specific soft tissues, membranes or cells and high specific bone cell binding and high activity for

10 disease condition's of the bone, or alternatively;

(d)   (iv)   independently selecting high binding and low activity in soft tissues, membranes or cells and in bone cells for use as antagonists in the medical treatment of hormonal disorders and cancers.

15   Subsequent different standard assays or toxicity determinations are performed on the amino acid analogs pursuant to identifying a useful pharmaceutical.

In one embodiment, in step (d) (i) the high binding and high activity in the specific tissues, membranes or

20 cells and a high specific bone cell binding and high activity are each about 50% or greater than that of the reference peptide.

In one embodiment, in step (d) (ii) the high binding and high activity as agonists for medical treatment in the

25 specific tissues, membranes, or cells is about 50% or greater than that of the reference peptide and the low specific bone cell binding is about 5 to 0% and low activity of bone cell is about 10% or less.

In one embodiment, in step (d) (iii) the low binding

30 is less than 10% and the low activity is about 5 to 0% in specific tissue, and high specific bone cell binding and the high activity is about 50% or greater, for disease conditions of the bone.

In one embodiment, in step (d) (iv) the high binding

35 is greater than 50% and the low activity is essentially 5 to 0% in soft tissues, membranes or cells or in bone, for use as pharmaceutical antagonists in the medical treatment of

hormonal disorders and cancers.

Preferably, the soft tissue etc. are derived from human or bovine kidey.

Thus, based on the present selection process (glu^6 or
5   ala^3)bPTH(1-34) amide is useful as an agonist for soft tissue or for bone, (d),(i).

Similarly, phe^{6}bPTH(1-34) amide is selected as being useful as a specific agonist for disease conditions in seoft tissue, etc., (d), (ii).

10   Similarly, phe^3 bPTH(1-34) amide is selected as being useful as a specific agonist for disease conditions in bone (d)(iii).

Also, ser^6 bPTH(1-34) amide is selected as an antagonist for disease conditions in soft tissue and bone
15   (d), (iv).

In summary, the present invention describes a model for the structures of PTH(1-34) and PTHrP(1-34) in which their biologically active conformations at the receptor consist of N- and C-terminal amphiphilic helices connected
20   by a loop of approximately 12 residues. Interhelical interactions result in a hydrophobic core with externally-facing hydrophilic residues that presumably include determinants of receptor binding and activation. The synthesis of analogues substituted in two such externally-
25   facing positions permits the demonstration that amino acid positions 3, 6 or 9 contribute important determinants of receptor binding and activation. Further delineation of the structural constraints at these positions will facilitate the rational design of potent PTH antagonists.

30   Specific embodiments of the present invention include the following wherein serine is at position 1 and the amino acid at position 3 is different. Group A' is independently selected from the remaining peptides of the 1-34 active unit of hPTH, pPTH, bPTH, or their Z=COOH or COO-+M or
35   -(C=O)NH$_2$ terminated derivatives; or the remaining 4-84 sequence of hPTH or bPTH or hPTHrP:
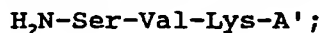
H$_2$N-Ser-Val-Lys-A';

H₂N-Ser-Val-Phe-A';
H₂N-Ser-Val-Leu-A';
H₂N-Ser-Val-Ala-A';
H₂N-Ser-Val-Thr-A';
5   H₂N-Ser-Val-Cys-A';
H₂N-Ser-Val-Tyr-A';
H₂N-Ser-Val-Asp-A';
H₂N-Ser-Val-Glu-A';
H₂N-Ser-Val-Asn-A';
10   H₂N-Ser-Val-Gln-A';
H₂N-Ser-Val-Lys-A';
H₂N-Ser-Val-Arg-A';
H₂N-Ser-Val-His-A';
H₂N-Ser-Val-Val-A';
15   H₂N-Ser-Val-Ile-A';
H₂N-Ser-Val-Trp-A';
H₂N-Ser-Val-Met-A';
H₂N-Ser-Val-Pro-A';
H₂N-Ser-Val-Nle-A';
20   H₂N-Ser-Val-D-Nal-A'; or
H₂N-Ser-Val-Orn-A'.

In all of the mofified peptide amino acid sequences
described herein, Z=amide is preferred. The Z=amide analog
is more preferred for the peptide sequences described in
25 any of the claims below.

In the analogues of the following list, the amino acid
at position 1 is alanine, and the amino acid at position 3
is varied:

H₂N-Ala-Val-Ala-A';
30   H₂N-Ala-Val-Thr-A';
H₂N-Ala-Val-Cys-A';
H₂N-Ala-Val-Tyr-A';
H₂N-Ala-Val-Asp-A';
H₂N-Ala-Val-Glu-A';
35   H₂N-Ala-Val-Asn-A';
H₂N-Ala-Val-Gln-A';
H₂N-Ala-Val-Lys-A';

H_2N-Ala-Val-Arg-A';

H_2N-Ala-Val-His-A';

H_2N-Ala-Val-Val-A';

H_2N-Ala-Val-Leu-A';

5　　H_2N-Ala-Val-Ile-A';

H_2N-Ala-Val-Pro-A';

H_2N-Ala-Val-Phe-A';

H_2N-Ala-Val-Trp-A';

H_2N-Ala-Val-Met-A';

10　　H_2N-Ala-Val-Nle-A';

H_2N-Ala-Val-D-Nal-A'; or

H_2N-Ala-Val-Orn-A'".

More preferred are these analogues are where A' is the 4-34 amino acid sequence of hPTH, especially those having

15　　derivatives terminating in $Z=-(C=O)-NH_2$.

Additional specific embodiments of the present invention include the following analogs wherein the amino acid at positions 1 and 3 are both serine, the amino acid at position 6 is replaced by Group J, and Group B' is

20　　independently selected from the remainder of the remaining peptides of the 7-34 active unit of hPTH, or bPTH, or pPTH, or their Z=COOH or COO-+M or amide derivative, or the 7-84 sequence of hPTH or bPTH or pPTH or hPTHrP:

H_2N-Ser-Val-Ser-Glu-Ile-Ala-B';

25　　H_2N-Ser-Val-Ser-Glu-Ile-Thr-B';

H_2N-Ser-Val-Ser-Glu-Ile-Cys-B';

H_2N-Ser-Val-Ser-Glu-Ile-Tyr-B';

H_2N-Ser-Val-Ser-Glu-Ile-Asp-B';

H_2N-Ser-Val-Ser-Glu-Ile-Glu-B';

30　　H_2N-Ser-Val-Ser-Glu-Ile-Asn-B';

H_2N-Ser-Val-Ser-Glu-Ile-Ser-B';

H_2N-Ser-Val-Ser-Glu-Ile-Lys-B';

H_2N-Ser-Val-Ser-Glu-Ile-Arg-B';

H_2N-Ser-Val-Ser-Glu-Ile-His-B';

35　　H_2N-Ser-Val-Ser-Glu-Ile-Val-B';

H_2N-Ser-Val-Ser-Glu-Ile-Leu-B';

H_2N-Ser-Val-Ser-Glu-Ile-Ile-B';

H_2N-Ser-Val-Ser-Glu-Ile-Pro-B';

H_2N-Ser-Val-Ser-Glu-Ile-Phe-B';

H_2N-Ser-Val-Ser-Glu-Ile-Trp-B';

H_2N-Ser-Val-Ser-Glu-Ile-Met-B';

5   H_2N-Ser-Val-Ser-Glu-Ile-Gly-B';

H_2N-Ser-Val-Ser-Glu-Ile-Nle-B';

H_2N-Ser-Val-Ser-Glu-Ile-D-Nal-B'; or

H_2N-Ser-Val-Ser-Glu-Ile-Orn-B'.

Additional specific embodiments of the present
10 invention include the following wherein Group B' is
selected from the remainder of the remaining peptides of
the 7-34 active unit of hPTH or bPTH or their Z=COOH or
COO-+M or amide derivative, or remaining 7-84 sequence of
hPTH, bPTH, pPTH, or hPTHrP. In the list of analogs which
15 follow the amino acid at position 1 is alanine and position
3 is serine, and position 6 is a variety of amino acids.

H_2N-Ala-Val-Ser-Glu-Ile-Ala-B';

H_2N-Ala-Val-Ser-Glu-Ile-Thr-B';

H_2N-Ala-Val-Ser-Glu-Ile-Cys-B';

20   H_2N-Ala-Val-Ser-Glu-Ile-Tyr-B';

H_2N-Ala-Val-Ser-Glu-Ile-Asp-B';

H_2N-Ala-Val-Ser-Glu-Ile-Glu-B';

H_2N-Ala-Val-Ser-Glu-Ile-Asn-B';

H_2N-Ala-Val-Ser-Glu-Ile-Ser-B';

25   H_2N-Ala-Val-Ser-Glu-Ile-Lys-B';

H_2N-Ala-Val-Ser-Glu-Ile-Arg-B';

H_2N-Ala-Val-Ser-Glu-Ile-His-B';

H_2N-Ala-Val-Ser-Glu-Ile-Val-B';

H_2N-Ala-Val-Ser-Glu-Ile-Leu-B';

30   H_2N-Ala-Val-Ser-Glu-Ile-Ile-B';

H_2N-Ala-Val-Ser-Glu-Ile-Pro-B';

H_2N-Ala-Val-Ser-Glu-Ile-Phe-B';

H_2N-Ala-Val-Ser-Glu-Ile-Trp-B';

H_2N-Ala-Val-Ser-Glu-Ile-Met-B';

35   H_2N-Ala-Val-Ser-Glu-Ile-Gly-B';

H_2N-Ala-Val-Ser-Glu-Ile-Nle-B'; or

H_2N-Ala-Val-Ser-Glu-Ile-D-Nal-B';

H$_2$N-Ala-Val-Ser-Glu-Ile-Orn-B'.

Additional specific embodiments of the present invention include the following wherein Group B' is selected from the remainder of the remaining peptides of the 7-34 active unit of hPTH or bPTH or pPTH, or their Z=COOH or COO-+M or amide derivative, or remaining 7-84 sequence of hPTH, bPTH, pPTH or hPTHrP:

H$_2$N-Ser-Val-Phe-Glu-Ile-Phe-B';

H$_2$N-Ser-Val-Phe-Glu-Ile-Ser-B';

H$_2$N-Ser-Val-Tyr-Glu-Ile-Phe-B';

H$_2$N-Ser-Val-Tyr-Glu-Ile-Ser-B';

H$_2$N-Ser-Val-Phe-Glu-Ile-Ala-B';

H$_2$N-Ser-Val-Lys-Glu-Ile-Ala-B';

H$_2$N-Ser-Val-His-Glu-Ile-Ala-B';

H$_2$N-Ser-Val-Leu-Glu-Ile-Ala-B';

H$_2$N-Ser-Val-His-Glu-Ile-Glu-B';

H$_2$N-Ser-Val-Leu-Glu-Ile-Glu-B';

H$_2$N-Ser-Val-Lys-Glu-Ile-Glu-B';

H$_2$N-Ser-Val-Phe-Glu-Ile-Glu-B';

H$_2$N-Ser-Val-Nle-Glu-Ile-Glu-B';

H$_2$N-Ser-Val-D-Nal-Glu-Ile-Glu-B';

H$_2$N-Ser-Val-Orn-Glu-Ile-Glu-B';

H$_2$N-Ala-Val-Phe-Glu-Ile-Phe-B';

H$_2$N-Ala-Val-Phe-Glu-Ile-Ser-B';

H$_2$N-Ala-Val-Tyr-Glu-Ile-Phe-B';

H$_2$N-Ala-Val-Tyr-Glu-Ile-Ser-B';

H$_2$N-Ala-Val-Phe-Glu-Ile-Ala-B';

H$_2$N-Ala-Val-Lys-Glu-Ile-Ala-B';

H$_2$N-Ala-Val-His-Glu-Ile-Ala-B';

H$_2$N-Ala-Val-Leu-Glu-Ile-Ala-B';

H$_2$N-Ala-Val-His-Glu-Ile-Glu-B';

H$_2$N-Ala-Val-Leu-Glu-Ile-Glu-B'; or

H$_2$N-Ala-Val-Lys-Glu-Ile-Glu-B'.

More preferred are those active 1-34 amino acid sequences of hPTH, especially those amino acid sequences terminating in Z=COOH or COO-+M or (C=O)NH$_2$.

In the analogues of the following list, the amino acid
at position 1 is serine, and the amino acid at position 3
is varied and the amino acid at position 6 is varied and
position 9 is His. Group D' is selected from the remainder

5 of the remaining amino acids of the 10-34 active unit of
hPTH, or bPTH or pPTH, or the Z=COOH or COO-+M or (C=O)NH$_2$
derivative or the remaining 10-84 amino acids of hPTH,
bPTH, pPTH, or hPTHrP:

    H$_2$N-Ser-Val-Phe-Glu-Ile-Gln-Leu-Met-His-D';

10   H$_2$N-Ser-Val-Phe-Glu-Ile-Ser-Leu-Met-His-D';

    H$_2$N-Ser-Val-Ser-Glu-Ile-Phe-Leu-Met-His-D';

    H$_2$N-Ser-Val-Ser-Glu-Ile-Ser-Leu-Met-His-D';

    H$_2$N-Ser-Val-Tyr-Glu-Ile-Gln-Leu-Met-His-D';

    H$_2$N-Ser-Val-Tyr-Glu-Ile-Ser-Leu-Met-His-D';

15   H$_2$N-Ser-Val-Phe-Glu-Ile-Phe-Leu-Met-His-D';

    H$_2$N-Ser-Val-Tyr-Glu-Ile-Phe-Leu-Met-His-D'; or

    H$_2$N-Ser-Val-Phe-Glu-Ile-Nle-Leu-Met-Phe-D'.

In the analogs of the following list the amino acid at
position 1 is serine, B^3 is serine and J is glutamine and

20 only the amino acid X at position 9 is different. D' is
defined above:

    H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Ala-D';

    H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Ser-D';

    H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Leu-D';

25   H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Phe-D';

    H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Tyr-D';

    H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Glu-D';

    H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Lys-D';

    H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Gln-D'; or

30   H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Nle-D.

In the amino acid analogues of the following list, the
amino acid at position 1 is alanine, the amino acid at
position 3 is serine, amino acid J at position 6 is
glutamine, and the only amino acid X at position 9 is

35 different. D' is defined above:

    H$_2$N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Ala-D';

    H$_2$N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Ser-D';

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H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Leu-D';

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Phe-D';

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Tyr-D';

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Glu-D';

5   H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Lys-D';

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Gln-D';

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Nle-D'; or

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-D-Nal-D.

In the analogues of the following list, the amino acid

10 at position 1 is alanine, and X is phe and only the amino

acids (B,J) at positions 3 and 6 are different. D' is

defined above:

H₂N-Ala-Val-Phe-Glu-Ile-Gln-Phe-Met-His-D';

H₂N-Ala-Val-Phe-Glu-Ile-Ser-Phe-Met-His-D';

15   H₂N-Ala-Val-Ser-Glu-Ile-Phe-Phe-Met-His-D';

H₂N-Ala-Val-Ser-Glu-Ile-Ser-Phe-Met-His-D';

H₂N-Ala-Val-Tyr-Glu-Ile-Gln-Phe-Met-His-D';

H₂N-Ala-Val-Tyr-Glu-Ile-Ser-Phe-Met-His-D';

H₂N-Ala-Val-Phe-Glu-Ile-Phe-Phe-Met-His-D';

20   H₂N-Ala-Val-Tyr-Glu-Ile-Phe-Phe-Met-His-D';

H₂N-Ala-Val-Nle-Glu-Ile-Phe-Phe-Met-His-D'; or

H₂N-Ala-Val-D-Nal-Glu-Ile-Phe-Phe-Met-Phe-D'.

DETAILED DESCRIPTION OF THE SYNTHESIS OF POLYPEPTIDES

The polypeptides of the present invention are prepared

25 by the synthesis techniques known in this art. The

teachings of the U.S. patents 4,318,905 and 3,531,258 are

specifically incorporated herein by reference. A key

feature of the present invention is the preparation of

biologically active synthetic polypeptides wherein at least

30 the 3 amino acid B, or the 6 amino acid, or the 9 amino

acid X, or combinations thereof are replaced a with

natural, unusual or synthetic amino acid analogue.

The polypeptides of the present invention may be

synthesized by any techniques that are known to those

35 skilled in the peptide art. An excellent summary of the

many techniques so available may be found in J.M. Stewart

and J.D. Young, "Solid Phase Peptide Synthesis", 2nd ed.,

Pierre Chem. Co., Rockford, IL., (1969) and J. Meinenhofer,
"Hormonal Proteins and Peptides," Vol. 2, p. 46, Academic
Press (New York), 1973 for solid phase peptide synthesis
and E. Schroder and K. Lubke, "The Peptides", Vol. 1,
5 Academic Press (New York), 1965 for classical solution
synthesis.

In general, these methods comprise the sequential
addition of one or more amino acids or suitably protected
amino acids to a growing peptide chain. Normally, either
10 the amino or carboxyl group of the first amino acid is
protected by a suitable protecting group. The protected or
derivatized amino acid can then be either attached to an
inert solid support or utilized in solution by adding the
next amino acid in the sequence having complimentary (
15 amino or carboxyl) group suitably protected, under
conditions suitable for forming the amide linkage. The
protecting group is then removed from this newly added
amino acid residue and the next amino acid (suitably
protected) is then added, and so forth. After all the
20 desired amino acids have been linked in the proper
sequence, any remaining protecting groups (and any solid
support) are removed sequentially or concurrently, to
afford the final polypeptide. By simple modification of
this general procedure, it is possible to add more than one
25 amino acid at a time to a growing chain, for example, by
coupling (under conditions which do not racemize chiral
centers) a protected tripeptide with a properly protected
dipeptide to form, after deprotection, a pentapeptide.

A particularly preferred method of preparing compound
30 of the present invention involves solid phase peptide
synthesis.

In this particularly preferred method the alpha-amino
function of the amino acids is protected by an acid or base
sensitive group. Such protecting groups should have the
35 properties of being stable to the conditions of peptide
linkage formation, while being readily removable without
destruction of the growing peptide chain or racemization of

any of the chiral centers contained therein. Suitable
protecting   groups   are   t-butyloxycarbonyl   (Boc),
benzyloxycarbonyl (Cbz), biphenylisopropyloxycarbonyl, t-
amyloxycarbonyl,   isobornyloxycarbonyl,   alpha,alpha-
5 dimethyl-3.5-dimethoxybenzyloxycarbonyl,   o-
nitrophenylsulfenyl,   2-cyano-t-butyloxycarbonyl,   9-
fluoroenylmethyloxycarbonyl and the like, especially t-
butyloxcarbonyl (Boc).

Particularly preferred side chain protecting groups
10 are,   for   arginine:nitro,   p-toluenesulfonyl,   4-
methoxybenzenesufonyl, Cbz, Boc and adamantyloxycarbonyl;
for   tyrosine:benzyl,   o-bromobenzyloxycarbonyl,   2,6-
dichlorobenzyl,   isopropyl,   cyclohexyl,   cyclopentyl   and
acetyl;   for   serine:benzyl   and   tetrahydropyranyl;   for
15 histidine; benzyl, p-toluenesulfonyl and 2,4-dinitrophenyl.

The C-terminal amino acid is attached to a suitable
solid support.   Suitable solid supports useful for the
above synthesis are those materials which are inert to the
reagents   and   reaction   conditions   of   the   stepwise
20 condensation-deprotection reactions,   as   well   as   being
insoluble in the media used.   Suitable solid supports are
chloromethylpolystyrene-divinylbenzene   polymer,
hydroxymethyl-polystyrene-divinylbenzene polymer, and the
like, especially chloromethyl-polystyrene-1% divinylbenzene
25 polymer.   For the special case where the C-terminus of the
compound will be glycinamide, a particularly useful support
is the benzhydrylaminopolystyrene-divinylbenzene polymer
described by P. Rivaille, et al. Helv. Chim. Acta., 54 2772
(1971).   The attachment to the chloromethyl polystyrene-
30 divinylbenzene type of resin is made by means of the
reaction of the N^{alpha}-protected amino acid, especially the
Boc-amino   acid,   as   its   cesium,   tetramethylammonium,
triethylammonium,   4,5-diazabicyclo[5.4.0]undec-5-ene,   or
similar   salt   in   ethanol,   acetonitrile,   N,N-
35 dimethylformamide (DMF),   and the   like,   especially the
cesium salt in DMF, with the chloromethyl resin at an
elevated temperature, for example between 40° and 60°C.,

preferably about 50°C., for from about 12 to 48 hours,
preferably about 24 hours. The $N^{|alpha|}$-Boc-amino acid is
attached to the benzhydrylamine resin by means of an N,N'-
dicyclohexylcarbodiimide (DCC)/1-hydroxybenzatriazole (HBT)

5   mediated coupling for from about 2 to about 24 hours,
preferably about 12 hours at a temperature of between about
10° and 50°C., preferably 25°C, in a solvent such as
dichloromethane or DMF, preferably dichlormethane. The
coupling of successive protected amino acids can be carried

10   out in an automatic polypeptide synthesizer as is well
known in the art. The removal of the N^a-protecting groups
may be performed in the presence of, for example, a
solution of trifluoroacetic acid in methylene chloride,
hydrogen chloride in dioxane, hydrogen chloride in acetic

15   acid, or other strong acid solution, preferably 50%
trifluoroacetic acid in dichloromethane at about ambient
temperature. Each protected amino acid is preferably
introduced in approximately 2.5 molar excess and the
coupling may be carried out in dichloromethane

20   dichloromethane/DMF mixtures. DMF and the like, especially
in methylene chloride at about ambient temperature. The
coupling agent is normally DCC in dichloromethane but may
be N,N'-di-iso-propylcarbodimide or other carbodiimide
either alone or in the presence of HBT, N-

25   hydroxysuccinimide, other N-hydroximides or oximes.
Alternately protected amino acid active esters (e.g., p-
nitrophenyl, pentafluorophenyl and the like) or symmetrical
anhydrides may be used.

     At the end of the solid phase synthesis, the fully

30   protected polypeptide is removed from the resin support is
of benzyl ester type, cleavage is by means of aminolysis
with alkylamine or fluoroalkylamine for peptides with a
proline C-terminus, or by aminolysis with, for example,
ammonia/methanol or ammonia/ethanol for peptides with a

35   glycine C-terminus at a temperature between 10° and 50°C.,
preferably about 25°C., for between about 12 and 24 hours
preferably about 18 hours. Alternatively, the peptide may

be removed from the resin by transesterification, e.g.,
with methanol, followed by aminolysis. The protected
peptide may be purified at this point by HPLC or by silica
gel chromatography.

5   The specific peptide synthesis and purfication steps
are described below in the Examples and are summarized in
Tables 2,3 and 4.

Utility

The modified peptide analogs of the present invention,
10   i.e. those PTH, etc. analogs having modified amino acids at
the 3,6 or 9 positions, or combinations of substitution at
the 3,6 and 9 positions, are useful as agonists or as
antagonists for treatment of medical conditions or diseases
involving parathyroid hormone, particularly in a human
15   being. The diseases etc. are described herein.

Administration

The exact doses and regimen for administration of
these compounds and compositions will necessarily be
dependent upon the needs of the individual subject being
20   treated, the type of treatment, the degree of affliction or
need and, of course, the judgment of the medical
practitioner. In general, parenteral administration
requires lower dosage than other methods of administration
which are more dependent upon absorption. However, certain
25   agents, such dimethyl sulfoxide, appear to enhance the
movement of polypeptide compounds through the skin.

Compositions

A further aspect of the present invention relates to
pharmaceutical compositions containing as an active
30   ingredient, a compound of the present invention the
compositions comprise a compound in admixture with a
pharmaceutically acceptable non-toxic carrier (excipient).
As mentioned above, such compositions may be prepared for
use for parenteral (subcutaneous, intramuscular or
35   intravenous) administration particularly in the form of
liquid solutions or suspensions; for use in vaginal or
rectal administration particularly in semisolid forms such

as creams and suppositories; for oral or buccal administration particularly in the form of tablets or capsules; or intranasally particularly in the form of powders, nasal drops or aerosols.

5   The compositions may conveniently be administered in unit dosage form and may be prepared by any of the methods well-known in the pharmaceutical art, for example as described in Remington's *Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pa., 1970. Formulations for
10   parenteral administration may contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Formulations for vaginal or rectal administration, e.g. suppositories, may contain as
15   excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for inhalation administration may be solid and contain as excipients, for example, lactose or may be aqueous or oily solutions for administration in the form of nasal drops. For buccal
20   administration excipients include sugars, calcium stearate, magnesium stearate, pregelinatined starch, and the like.

It is often desirable to deliver the compounds of the present invention to the subject over prolonged periods of time, for example, for periods of one week to one year from
25   a single administration. Various slow release, depot or implant dosage forms may be utilized. For example, a dosage form a may contain a pharmaceutically acceptable non-toxic salt of the compounds which has a low degree of solubility in body fluids, for example, (a) an acid
30   addition salt with a polybasic acid such as phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a salt with a polyvalent metal
35   cation such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from e.g., N,N'-dibenzyl-

ethylenediamine or ethylenediamine; or (c) combinations of
(a) and (b) e.g. a zinc tannate salt. Additionally, the
compounds of the present invention or, preferably, a
relatively insoluble salt such as those just described, may

5   be formulated in a gel, for example, an aluminum
monostearate gel with, e.g. sesame oil, suitable for
injection. Particularly preferred salts are zinc salts,
zinc tannate salts, pamoate salts, and the like. Another
type of slow release depot formulation for injection would

10 contain the compound or salt dispersed for encapsulated in
a slow degrading, non-toxic, non-antigenic polymer such as
a polylactic acid/polyglycolic acid polymer for example as
described in U.S. Pat. No. 3,773,919. The compounds or,
preferably, relatively insoluble salts such as those

15 described above may also be formulated in cholesterol
matrix silastic pellets, particularly for use in animals.
Additional slow release, depot or implant formulations,
e.g. liposomes, are well known in the literature. See, for
example, "Sustained and Controlled Release Drug Delivery

20 Systems", J.R. Robinson ed., Marcel Dekker, Inc., New York,
1978.

The following Examples are presented for the purpose
of description and illustration only. They are not to be
construed as limiting in any way.

25 MATERIALS--Synthetic bovine PTH(1-34) was obtained
from Bachem, Inc., Torrance, California. Synthetic human
PTHrP(1-34)amide was obtained from Merck Sharp and Dohme,
West Point, Pennsylvania. Synthetic bovine [Tyr^{34}]PTH(7-
34)amide and [Tyr^{34}]PTH(7-34)amide were obtained from

30 Peninsula Laboratories, Inc., Belmont, California. The
purity of these peptides exceeded 95% by HPLC. Appropriate
peptide composition was verified by quantitative amino-acid
analysis.

CD SPECTROSCOPY. Far ultraviolet circular dichroism

35 (CD) spectra were obtained in phosphate buffer (ph 7.0) at
25±1°C in a 1.0 mm cell on an Instruments SA Jobin Yvon
circular dichrograph calibrated with (+) 10-camphorsulfonic

acid and epiandosterone. Peptides were analyzed at
concentrations ranging from 0.1-0.3 mg/ml. The mean molar
ellipticity per residue at 222 nm ([θ]222 nm-, deg·cm^2/dmol)
was used to derive estimates of alpha-helicity according to
5   the method of Taylor and Kaiser (ref. 29).

PEPTIDE SYNTHESIS AND PURIFICATION--Peptide synthesis
was carried out using an Applied Biosystems Model 430A
Peptide Synthesizer, Foster City, California. A t-Boc-Phe-
OCH2- Pam resin was used as the solid support, and the
10   following   t-Boc   (tert-butyloxycarbonyl)   amino   acid
derivatives were employed: Arg(Tos), Asp(OBzl), Glu(OBzl),
His(DNP), His(Z), Lys(Cl-Z), Ser(Bzl), Thr(Bzl), Trp(CHO),
and Tyr(Br-Z).   A standard program (pre-formed symmetric
anhydrides and pre-formed HOBt esters) with inclusion of
15   capping cycles were used. The general synthesis of the
peptides is found, for example, in U.S.Patent No. 4,318,905
which is specifically incorporated herein by reference in
its entirety.

The following Examples are only provided to be
20   descriptive and explanatory. They are not to be construed
to be limiting in any way.

Variations in synthesis are described herein below and
summarized in Tables 2,3 and 4.

EXAMPLE 1

25   PEPTIDE PREPARATION

Standard t-Boc cycles exist for the activation,
solvent exchange, and coupling processes; called ACT, CONC,
and RV cycles, respectively.   The set of vessel cycl
assignments in a standard t-Boc static run file is
30   designated for each amino acidon the basis of testing and
synthesis optimization performed at Applied biosystems.

Descriptions of the cycles assigned in the static run
are described later under "Activator Cycles (ACT)",
"Concentrator Cycles (CONC)", and Reaction Vessel Cycles
35   (RV)".   Other vessel cycles for synthesis are also
included.

All amino acids with the exception of Arg, Asn, and

Gln are activated to symmetric anhydride using 1 mmol of
0.5 M DCC in DCM. The by-product of this reaction, DCU,
begins to precipitate almost immediately. The total
activation time allotted for each cycle is eight minutes.
5   Four of these eight minutes are for purging excess DCM from
the solution, allowing approximately 2 mL of DCM to be
removed. The purging also cools the solution which aids
the DCU precipitation.

When the activation is complete the amino acid
10 solution is transferred from the ACT to the CONC. A
complete wash of the transfer line proceeds and follows
each transfer between vessels. This procedure ensures that
the line is clean both before and after the transfer, and
eliminates the possibility of amino acid carry-over between
15 cycles.

Transfer times of the activated amino acid solution to
the CONC are longer for aboc 4 cycle than for the aboc 1,
aboc 2 or aboc 3; again because of the larger solution
volume handled in the aboc 4 ACT cycle. Two DCM rinses of
20 the ACT are transferred into the CONC in all single couple
cycles. The time listed in Table 2 includes the initial
activation time and purging time.

At the end of the activation and purging, the amino
acid solution is ready for transfer to the CONC. A
25 complete wash of the transfer line precedes and follows
each transfer between vessels. This procedure ensures that
the line is clean both before and after the transfer to
eliminate the possibility of carry-over between cycles.
The activated amino acid solution is transferred to the
30 CONC along with one subsequent DMF rinse of the ACT.

The HOBt-ester activation cycles produce twice as much
DCU as the symmetric anhydride cycles; therefore, a more
extensive washing process is required after the transfer.
This washing consists of two 50:50 DCM:MeOH washes followed
35 by three DCM washes. The first DCM:MeOH wash almost fills
the vessel; any DCU carried to the top of the vessel during
the purging will be dissolved in this wash. The second

DCM:MeOH solution ensures the removal of any DCU adhering to the frit. The residual MeOH is rinsed away with three subsequent DCM washes.

TABLE 2

SINGLE COUPLE ACTIVATOR CYCLES

CYCLE NAME	DESIGNATED AMINO ACID	DISSOLVING SOLVENT (about)	DISSOLVING TIME (MIN.) (about)	TRANSFER TIMES* (Sec)
aboc 1	Ala,Asp(OBzl), Cys(4-Me-Bzl), Cys(p-OMeBzl) Glu(OBzl), Gly, Ile,Met, Phe, Ser(Bzl), Thr(Bzl), Tyr(Br-Z), Val	3mL DCM	2	1=30 2=23
aboc 2	Lys(Cl-Z), Pro His(Tos)-dry	3 mL DCM	8	1=35 2=23
aboc 3	Leu, Met(O), Trp, Trp(CHO)	0.3 mL DMF 2.5 mL. DCM (2 separate DCM deliveries)	7	1=30 2=23
aboc 4**	His(Tos)	Pre-dissolved in 7 mL DCM	0	1=40

* 1=Transfer from amino acid cartridge to the Activator
   2=Transfer from the Activator to the Concentrator

** aboc 4 is used to His(Tos)DCHA after the completion of the ion exchange procedure

TABLE 3

DOUBLE COUPLING ACTIVATOR CYCLES

CYCLE NAME	AMINO ACID	DISSOLVING SOLVENT	DISSOLVING TIME	ACTIVATED DERIVATIVE	ACTIVATION TIME
aboc ld	Asn,Gln	4.0 mL HOBt 0.3 mL DCM	6.5 min	HOBt Ester	33 min
aboc 2d	Arg(Tos)	4.0 mL HOBt 1.5 mL DCM	8 min	HOBt Ester	33 min
aboc 3d	Arg(MTS)	4.0 mL HOBt 1.5 mL DCM	8 min	HOBt Ester	48 min
aboc 4d	His(Tos)	Predissolved in 7 mL DCM	0 min	Symmetric Anhydride	8 min

** aboc 4d is used to double couple His(Tos)DCHA after the completion of the ion exchange procedure

TABLE 4

CONCENTRATOR CYCLES
PURGING SCHEME

CYCLE NAME	AMINO ACIDS	TOTAL TIME	PURGE INTERVALS	DMF ADDITION (about)	HEATER SETTING/ USAGE
Single couple:					
cboc 1	Ala	13 min	4 min 5 min 4 min	2 mL 2 mL	15°C/turned on after 1st DMF delivery
cboc 2	Asp(OBzl), Cys(4me-Bzl), Glu(OBzl),Ile, Met, Met(O), Phe, Pro, Ser(Bzl) Thr(Bzl), Trp**, Val	16 min	10 min 6 min	2mL 2mL	15°C/turned on after 1st DMF Cys(p-MeBzl), delivery
cboc 3	Leu Trp(CHO)**	14 min	10 min 4 min*	1 mL 3 mL	5°C/turned on after 1st DMF delivery
cboc 4	Lys(Cl-Z) Tyr(Br-Z)	6 min	5 min* 1 min*	1 mL 3 mL	15°C/turned on immediately
cboc 5	Gly, His (Tos)	6.5 min 0.5 min	6 min ---	1 mL	10°C/turned on immediately

TABLE 4 (Continued)

CYCLE NAME	AMINO ACIDS	TOTAL TIME	PURGE INTERVALS	DMF ADDITION (about)	HEATER SETTING/ USAGE
Double Couple:					
cboc 1d	Arg(Tos) Arg(MTS) ASN, Gln	0 min	0 min	---	10°C/heater not used
cboc 2d	His(Tos)	6.5 min	6 min 0.5 min	1 mL ----	10°C/heater not used
Recouple:					
c recpl	All single coupled amino acids	0 min	0 min	----	10°C/heater not used

*   Multiple short purges
**   Heater temperature setting is 25°C

The c recouple cycle is present on the standard, but
is not assigned in the standard t-Boc run. This cycle is
simply a transfer cycle designed to recouple any amino acid
using DCM as the coupling solvent. This cycle does not
5   include purging or heater use because the initial solvent
DCM, is not exchanged for a different coupling solvent,
DMF. The amino acid solution is transferred to the RV
along with two additional DCM rinses of CONC.

The c recpl cycle should be used in conjunction with
10 the normal single couple ACT cycle, and either recpl2 or
recpl2r, the DCM RV recoupled cycles.

EXAMPLE 2

REACTION VESSEL (RV) CYCLES

There are two types of RV cycles; those that take
15 resin sample and those that do not. Resin-sampling cycles
rinse the sampling line several times from the lower RV
valve block up through the bulkhead fitting and into the
RV. This rinse prevents accumulation of resin, TFA, or
coupling solution in the line.

20   Resin-sampling cycles have an "r" at the end of the
cycle name (i.e., rboc 1r). If the cycle has "r" at the
end (i.e., rboc 1rr), then two resin samples are taken
after the coupling. The "rr" cycles are included on this
disk for installation purposes. The RV cycles with the
25 same number designation, such as rboc 1 and rboc 1r, are
the same cycles, except that the resin sampler is used in
the latter cycles.

EXAMPLE 3

Single Couple Cycles -- rboc 1, 2, 1r, 2r, 1rr, 2rr

30   All the single couple RV cycles conform to the
following pattern:

1.   33% TFA in DCM for 80 seconds
2.   50% TFA in DCM for 18.5 minutes
3.   Three DCM washes
35 4.   10% DIEA in DMF for 1 minute
5.   10% DIEA in DMF for 1 minute
6.   Five DMF washes

7.   Coupling period

8.   Five DCM washes

There are two versions of single coupling RV cycles; these vary only in the length of the coupling period, the time when the deprotected resin-bound peptide is in solution with the activated amino acid. This period begins after "Ready to Receive" step and ends when the amino acid solution is drained from RV. The _rboc 1_ cycle and its associated resin-sampling cycles, _rboc 1r_.

Five extensive DMF washes ensure the complete removal of DIEA from the resin RV, and associates lines prior to coupling. The RV is now ready to receive the coupling solution from the CONC, so that the coupling period may ensue.

There are five DCM washes immediately following the coupling period to wash away uncoupled amino acid and DMF. As a secondary function, these washes provide a sampling solution. If one resin sample is taken, at last wash provides the medium for the sample. The double resin-sampling cycles (i.e., "rr" cycles) use the third and fifth wash to collect the two resin samples.

EXAMPLE 4

Double Couple Cycles -- rboc, 1d, 2d, 1dr, 1dr

All the double couple RV cycles use the following pattern:

1.   33% TFA in DCM for 80 seconds

2.   50% TFA in DCM for 18.5 minutes

3.   Three DCM washes

4.   10% DIEA in DMF for 1 minute

5.   10% DIEA in DMF for 1 minute

6.   Five DMF washes

7.   First coupling period

8.   Three DMF washes

9.   10% DIEA in DMF for 45 seconds

10.   One DMF wash

11.   Three DCM washes

    - End of First Half -

12. Second coupling period

13. One DMF wash

14. Five DCM washes

The first half of the doubling couple cycles are
5 similar to the single couple cycles. The first six
processes in the list above are exactly the same as the
equivalent processes in a single couple RV cycle. However,
there is a longer total coupling time for the double couple
cycles. The _rboc 1d_ and _rboc 1dr_ cycles each have a 42-
10 minute (first) coupling period; the _rboc 2d_ and _rboc 2dr_
cycles each have a 26-minute (first) coupling period.

Another difference appears during the washes
immediately following the coupling phase. Three DMF washes
remove any extra amino acid and then the resin is treated
15 with one more base.

Peptide resins were treated with thiophenol in
dimethylformamide to deblock histidine residues (ref. 30).

EXAMPLE 5

DEPROTECTION OF THE DNP GROUP OF
20   HISTIDINE FROM A PEPTIDE-RESIN

Suspend the peptide-resin in the reaction vessel in
the minimum amount of purified DMF needed to slurry the
resin (about 5 ml. per g resin). Add 20 mol of thiophenol
(0.102 mL/mmol) for each mole Dnp histidine present. Rock
25 the vessel at ambient temperature for one hour. Thiolysis
is rapid and probably complete in fifteen minutes. Wash
the resin thoroughly with DMF, water, EtOH, and DCM and dry
the peptide-resin. The peptide-resin is then cleaved in
the usual way. A small amount of intensely yellow Dnp-
30 thiophenol is usually absorbed to the resin and is
extracted with the peptide after cleavage. It is easily
removed from the peptide in the standard purification
procedures.

EXAMPLE 6

35   DEPROTECTION AND CLEAVAGE

The peptides are contacted with HF:anisole:DMS(10:1:1)
at -5 to 0°C for 6 minutes.

After removal of the N-terminal t-Boc protecting

group, peptides were deblocked and cleaved from the resin with anhydrous hydrogen fluoride.

EXAMPLE 7

EXTRACTION PROCEDURE

5   After the peptide-resin has reacted in HF at -5° to 0°C for 50 minutes, the HF is easily evaporated with the aid of nitrogen flow within ten to fifteen minutes. To prevent side reactions during this process, it is important to keep the reaction vessel at -5°C to 0°C. After removal

10   of the HF, ether is added to the reaction vessel and the peptide-resin-scavenger mixture is mixed for about 30 seconds. The ether solution is then filtered through a sintered glass funnel. Repeated two more time, this ether wash removes most of the scavengers.

15   The peptide is extracted from the peptide-resin mixture by stirring the mixture in 30% acetic acid. For those peptides not soluble in 30% acetic acid, a higher concentration of acetic acid is recommended. Typically, for one gram of peptide resin approximately 30 mL of 30%

20   acetic acid is used. The acetic acid extract is filtered through the same sintered glass funnel used to the ether extraction, but into a different filter flask. To ensure complete extraction of the peptide, repeat the extraction procedure using approximately 30 mL of 10% acetic acid (2

25   times). Dilute the acetic acid solution with water before freeze drying. A more dilute solution of acetic acid in water will remain frozen while a concentrated solution of acetic acid in water may melt during lyophilization.

The solution is then lyophilized to obtain crude

30   peptide.

For peptides containing serine, the N-O shift may occur during HF cleavage. Remaining formyl groups were eliminated by treatment with 1 M ethanolamine in 6 M guanidine-HCl at 0°C for 5 min.

35   ## EXAMPLE 8

DEFORMYLATION OF PEPTIDE

The formyl (CHO) protecting group of tryptophan is an

HF-trifluoromethane sulfonic acid stable species. Deformylation of a peptide synthesized by using TRP (CHO) requires a separate step after strong acid cleavage. Since the deformylation of Trp (CHO) can pose problems to even

5   with the most skilled the following procedure should be strictly followed.

1.   Dissolve the peptide containing Trp (CHO) in 6M guanidine HCl to produce a concentration between 1 and 10 mg/ml.

10   2.   Perform a UV spectral analysis on the dissolved peptide. For a peptide containing Trp (CHO), the 300 nm absorbance is greater than the 280 nm absorbance.

3.   Cool the peptide-guanidine solution in an ice bath. Using a magnetic stirring bar, stir the solution

15   until 0°C is reached.

4.   Add enough ethanolamine to the peptide-guanidine solu- solution to produce a final concentration of 1M. (1 mL ethanolamine/16.6 mL peptide-guanidine produces a 1M solution in ethanolamine). After the ethanolamine is

20   added, the pH of the solution should be between 10 and 11.

5.   Stir for five minutes at 0°C and then quench by adding concentrated HCl until the pH reaches 7.

6.   Perform a UV spectral analysis on the peptide to ensure complete deformylation of the tryptophan. The 280

25   nm absorbance is greater for the peptide containing the unprotected Trp than the 300 nm absorbance.

7.   The peptide can be desalted on a gel filtration column or dialyzed to remove the guanidine and ethanolamine. Alternatively, the solution is loaded onto

30   a preparative high performance liquid chromatography (HPLC) system for purification.

8.   Precautions

a.   Perform a "test" deformylation on 1 mg of peptide.

35   b.   After dissolving the peptide, chromatograph on an appropriate HPLC system. Column: C-8, Buffer A: 0.1% TFA; Buffer B: 0.1% TFA/60% CH_3CN; gradient: 0-100% B in 45

minut s.

c.   After deformylation, rechromatograph to
ensure product integrity. The deformylated product should
have a slightly shorter retention time.

EXAMPLE 9

DESALTING AND PURFICATION

The deformylated crude peptide preparations were
desalted on a reverse-phase column (Delta Pak C_{18}, 300 A°)
using a gradient of acetonitrile in 0.1% trifluoroacetic
acid (0-60%, 24 ml/min) followed by cation-exchange
chromatography on a TSK 535 CM column (7.5 x 150 mm) eluted
with a NaCl gradient (0 - 0.5 M) in 20 mM sodium phosphate
buffer, pH 6.4.

Final purification was achieved by reverse-phase high
performance liquid chromatography (HPLC) on a Vydac column
(218TP1022) (0.22 x 25 cm) with a 25 - 35% acetonitrile
gradient in 0.1% trifluoroacetic acid, flow rate 10 ml/min.
The progress of purification was followed using both plasma
desorption mass spectrometry (31) and analytical HPLC. The
final purity of peptides was greater than 95%. Appropriate
peptide composition was verified by quantitative amino-acid
analysis.

EXAMPLE 10

ION - EXCHANGE HPLC

Refer to M. Carlquist et al., (1984) J. of
Chromatography, Vol. 296, pp. 143-151 for general
procedure. A more specific procedure follows:

The instrument for the ion-exchange HPLC consisted of
an LKB 2150 HPLC pump, an LKB 2152 HPLC controller, LKB
2040 gradient-mixing valve, an LKB 2154 HPLC injector and
an LKB 2151 HPLC variable-wavelength detector connected to
an LKB 2210 recorder. The separation was carried out on an
LKB UltroPac TSK 535 CM cation-exchanger column (150 x 7.5
mm I.D.) Elution was performed with a gradient of sodium
chloride (0-0.3 M) in a sodium phosphate buffer, pH 6.4
(1.14 g sodium hydroxide and 22.5 ml 1 M phosphoric acid,
and water added to a final volume of 1000 ml), filtered

through a Millipore MF-filter (0.22 micro m) and degassed
by vacuum. The flow-rate was set at 1ml/min and the
absorbance of the eluent was recorded at 215 nm. Fractions
of 2 ml each were collected with an LKB 2112 RediRac
5   fraction collector and evaluated. Purification as obtained
on a reverse phase HPLC column.

EXAMPLE 11

PTH/PTHrP RECEPTOR BINDING AND ADENYLATE CYCLASE ASSAYS

10   Receptor binding assays were carried out in canine
renal plasma membranes and in UMR 106-H5 rat osteosarcoma
cells as previously described in detail (Ref. 14,32), using
^{125}I-labeled PTH(1-34)amide as the radioligand. Binding
potency (IC_{50}) was quantified as the concentration of
15   unlabeled peptide required for half-maximal displacement of
the radioligand. Non-specific binding, defined as residual
radioligand binding in the present of a greater than 1,
microM unlabeled bPTH(1-34), was subtracted from all
binding values. The ratio of specific:non-specific binding
20   of ^{125}I-PTHrP(1-34)amide average >10:1 and 3:1 in the renal
membrane, and UMR 106-H5 assays, respectively. Adenylate
cyclase activity was assessed in canine renal membranes by
the conversion of $[\alpha-32_p]$ATP to $[^{32}P]$cyclic AMP, essentially
as described, except for the addition of 100 micromolar GTP
25   (Ref. 33,34). Adenylate cyclase activity in UMR 106-H5
cells was assessed essentially as described (Ref. 35). In
brief, cells were incubated with [3H]adenine 1 micro Ci/ml
of serum-free MEM for two hours at 37°C to label the
endogenous ATP pool. Cells were then exposed to 0.4 mM
30   IBMX for 10 min, followed by the appropriate peptide for an
additional 10 min at ambient temperature. The cells are
then scraped in 20% TCA, and [3H]cyclic AMP is isolated by
the column procedure of Salomon et al. (Ref. 36). Potency
in the adenylate cyclase assays (Ka) is the concentration
35   of peptide producing half-maximal enzyme activation.

     While only a few embodiments of the invention have
been shown and described herein, it will become apparent to
those skilled in the art that various modifications and

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changes can be made in the replacement of the amino acids at 3 and/or 6 and/or 9 positions to produce polypeptide analogs that have agonist or antagonist pharmacological activity without departing from the spirit and scope of the
5   present invention.   All such modifications and changes coming within the scope of the appended claims are intended to be carried out thereby.

<u>WE CLAIM</u>

1.   A pharmaceutical composition comprising a compound of the formula:

H_2N-(Ser or Ala)^1-Val-B^3-Glu-Ile-J^6-(Leu or Phe)^7-Met-X^9-Asn^{10}-Leu-Gly-Lys-His-Leu-(Asn or Ser)^{16}-Ser-(Met or Leu)^{18}-

5   Glu-Arg^{20}-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp^{30}-Val-His-Asn-Phe^{34}-Z (Structure I), or

the pharmaceutically acceptable salts thereof, wherein:

the amino acid B at position 3 is independently
10 selected from L-serine or those other natural or synthetic D or L amino acids having a spatial volume comparable to or greater than serine not glycine,

the amino acid J at position 6 is independently selected from L-glutamine or from other natural or
15 synthetic D or L amino acids,

the amino acid X at position 9 is independently selected from L-histidine or from other natural synthetic D or L amino acids,

with the proviso that when group B is L-serine, and J
20 is L-glutamine, group X is not L-histidine, when group B is L-serine and group X is L-histidine, group J is not L-glutamine, and when group J is L-glutamine and group X histidine, group B is not L-serine, and

Z is independently selected from -COOH, -COO-+M
25 wherein M+ is selected from pharmaceutically acceptable cations, -(C=O)NH_2, or the sequence of amino acids of human, bovine or porcine parathyroid hormone (35-84) or human, bovine or porcine parathyroid hormone-related protein (PTHrP) (35-141).

2.   The pharmaceutical composition of Claim 1 wherein the amino acid at position 1 is serine, the amino acid at position 7 is leucine the amino acid at position 16 is Asn, and the amino acid at position 18 is methionine, and Z is
5 the sequence of amino acids for human PTH (35-84).

3.   The pharmaceutical composition of Claim 1 wherein the amino acid at position 1 is alanine, the amino acid at

position 7 is phenylalanine, the amino acid at position 16 is serine and the amino acid at position 18 is methionive, and Z is the sequence of amino acids for bovine PTH (35-84).

4. The pharmaceutical composition of Claim 1 wherein structure 1 the amino acid at position 1 is serine, the amino acid at position 7 is leucine, the amino acid at position 16 is serine, the amino acid at position 18 is leucine and Z is the remaining amino acid of porcine PTH (35-84).

5. The pharmaceutical composition of Claim 1 wherein Z is -COOH or COO-+M, or -(C=O)NH₂.

6. The pharmaceutical composition of Claim 5 wherein the amino acid at position 1 is serine, the amino acid at position 7 is leucine the amino acid at position 16 is Asn, and the amino acid at position 18 is methionine, and Z is COOH or COO-+M or -(C=O)NH₂.

7. The pharmaceutical composition of Claim 5 wherein the amino acid at position 1 is alanine, the amino acid at position 7 is phenylalanine, the amino acid at position 16 is serine and the amino acid at position 18 is leucine, and Z is COOH or COO-+M or -(C=O)NH₂.

8. The pharmaceutical composition of Claim 5 wherein in structure 1 the amino acid at position 1 is serine, the amino acid at position 7 is leucine, the amino acid at position 16 is serine, the amino acid at position 18 is leucine and Z is COOH or COO-+M or -(C=O)NH₂.

9. The pharmaceutical composition of Claim 5 wherein B is L-serine.

10. The pharmaceutical composition of Claim 9 wherein J is independently selected from Leu, Phe, Ala, Glu, Ser or Phe.

11. The pharmaceutical composition of Claim 5 wherein J is L-glutamine.

12. The pharmaceutical composition Claim 11 wherein B is independently selected from Ala, Phe, Gln, Glu, Lys, His, or Tyr.

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13. The pharmaceutical composition of Claim 5 wherein B is independently selected from L-Serine, Ala, Phe, Gln, Glu, Lys, His or Tyr.

14. The pharmaceutical composition of Claim 5 wherein B is independently selected from L-glutamine, Leu, Phe, Ala, Glu, Ser or Phe.

15. A pharmaceutical composition comprising a polypeptide independently selected from:

H_2N-Ser-Val-Lys-A';

H_2N-Ser-Val-Phe-A';

5 H_2N-Ser-Val-Leu-A';

H_2N-Ser-Val-Ala-A';

H_2N-Ser-Val-Thr-A';

H_2N-Ser-Val-Cys-A';

H_2N-Ser-Val-Tyr-A';

10 H_2N-Ser-Val-Asp-A';

H_2N-Ser-Val-Glu-A';

H_2N-Ser-Val-Asn-A';

H_2N-Ser-Val-Gln-A';

H_2N-Ser-Val-Lys-A';

15 H_2N-Ser-Val-Arg-A';

H_2N-Ser-Val-His-A';

H_2N-Ser-Val-Val-A';

H_2N-Ser-Val-Ile-A';

H_2N-Ser-Val-Trp-A';

20 H_2N-Ser-Val-Met-A';

H_2N-Ser-Val-Pro-A';

H_2N-Ser-Val-Nle-A';

H_2N-Ser-Val-D-Nal;

H_2N-Ser-Val-Orn;

25 ―――――――――――――――

H_2N-Ala-Val-Ala-A';

H_2N-Ala-Val-Thr-A';

H_2N-Ala-Val-Cys-A';

H_2N-Ala-Val-Tyr-A';

30 H_2N-Ala-Val-Asp-A';

H_2N-Ala-Val-Glu-A';

63

H$_2$N-Ala-Val-Asn-A';
H$_2$N-Ala-Val-Gln-A';
H$_2$N-Ala-Val-Lys-A';
H$_2$N-Ala-Val-Arg-A';
5   H$_2$N-Ala-Val-His-A';
H$_2$N-Ala-Val-Val-A';
H$_2$N-Ala-Val-Leu-A';
H$_2$N-Ala-Val-Ile-A';
H$_2$N-Ala-Val-Pro-A';
10   H$_2$N-Ala-Val-Phe-A';
H$_2$N-Ala-Val-Trp-A';
H$_2$N-Ala-Val-Met-A';
H$_2$N-Ala-Val-Nle-A';
H$_2$N-Ala-Val-D-Nal-A'; or
15   H$_2$N-Ala-Val-Orn-A';

where A' is selected from the 4-34 amino acid sequence
for human PTH or bovine PTH or their Z=-COOH or -COO+-M or
-(C=O)NH$_2$ derivatives, or the 4-84 sequence of hPTH, bPTH,
or pPTH or hPTHrP;

20   H$_2$N-Ser-Val-Ser-Glu-Ile-Ala-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Thr-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Cys-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Tyr-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Asp-B';
25   H$_2$N-Ser-Val-Ser-Glu-Ile-Glu-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Asn-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Ser-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Lys-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Arg-B';
30   H$_2$N-Ser-Val-Ser-Glu-Ile-His-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Val-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Leu-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Ile-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Pro-B';
35   H$_2$N-Ser-Val-Ser-Glu-Ile-Phe-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Trp-B';
H$_2$N-Ser-Val-Ser-Glu-Ile-Met-B';

H₂N-Ser-Val-Ser-Glu-Ile-Gly-B';
H₂N-Ser-Val-Ser-Glu-Ile-Nle-B';
H₂N-Ser-Val-Ser-Glu-Ile-D-Nal-B';
H₂N-Ser-Val-Ser-Glu-Ile-Orn-B';

5

H₂N-Ala-Val-Ser-Glu-Ile-Ala-B';
H₂N-Ala-Val-Ser-Glu-Ile-Thr-B';
H₂N-Ala-Val-Ser-Glu-Ile-Cys-B';
H₂N-Ala-Val-Ser-Glu-Ile-Tyr-B';

10   H₂N-Ala-Val-Ser-Glu-Ile-Asp-B';
H₂N-Ala-Val-Ser-Glu-Ile-Glu-B';
H₂N-Ala-Val-Ser-Glu-Ile-Asn-B';
H₂N-Ala-Val-Ser-Glu-Ile-Ser-B';
H₂N-Ala-Val-Ser-Glu-Ile-Lys-B';

15   H₂N-Ala-Val-Ser-Glu-Ile-Arg-B';
H₂N-Ala-Val-Ser-Glu-Ile-His-B';
H₂N-Ala-Val-Ser-Glu-Ile-Val-B';
H₂N-Ala-Val-Ser-Glu-Ile-Leu-B';
H₂N-Ala-Val-Ser-Glu-Ile-Ile-B';

20   H₂N-Ala-Val-Ser-Glu-Ile-Pro-B';
H₂N-Ala-Val-Ser-Glu-Ile-Phe-B';
H₂N-Ala-Val-Ser-Glu-Ile-Trp-B';
H₂N-Ala-Val-Ser-Glu-Ile-Met-B';

25   H₂N-Ala-Val-Ser-Glu-Ile-Gly-B';
H₂N-Ser-Val-Phe-Glu-Ile-Phe-B';
H₂N-Ser-Val-Phe-Glu-Ile-Nle-B';
H₂N-Ser-Val-Phe-Glu-Ile-D-Nal-B';
H₂N-Ser-Val-Phe-Glu-Ile-Orn-B';

30   H₂N-Ser-Val-Phe-Glu-Ile-Ser-B';
H₂N-Ser-Val-Tyr-Glu-Ile-Phe-B';
H₂N-Ser-Val-Tyr-Glu-Ile-Ser-B';
H₂N-Ser-Val-Phe-Glu-Ile-Ala-B';
H₂N-Ser-Val-His-Glu-Ile-Ala-B';

35   H₂N-Ser-Val-Leu-Glu-Ile-Ala-B';
H₂N-Ser-Val-Lys-Glu-Ile-Ala-B';
H₂N-Ser-Val-His-Glu-Ile-Glu-B';

H$_2$N-Ser-Val-Leu-Glu-Ile-Glu-B';

H$_2$N-Ser-Val-Lys-Glu-Ile-Glu-B';

H$_2$N-Ala-Val-Phe-Glu-Ile-Phe-B'

5   H$_2$N-Ala-Val-Phe-Glu-Ile-Ser-B';

H$_2$N-Ala-Val-Val-Glu-Ile-Phe-B';

H$_2$N-Ala-Val-Tyr-Glu-Ile-Phe-B';

H$_2$N-Ala-Val-Tyr-Glu-Ile-Ser-B';

H$_2$N-Ala-Val-Phe-Glu-Ile-Ala-B';

10   H$_2$N-Ala-Val-Lys-Glu-Ile-Ala-B';

H$_2$N-Ala-Val-His-Glu-Ile-Ala-B';

H$_2$N-Ser-Val-His-Glu-Ile-Ala-B'; or

H$_2$N-Ser-Val-Leu-Glu-Ile-Ala-B';

wherein Group B' is selected from the remainder of the

15 remaining peptides of 7-34 active unit of hPTH, or bPTH, or
pPTH, or their Z=COOH or COO-+M or -(C=O)NH$_2$ derivatives, or
the 7-84 sequence of hPTH or bPTH or hPTrH;

H$_2$N-Ser-Val-Phe-Glu-Ile-Gln-Leu-Met-His-D';

20   H$_2$N-Ser-Val-Phe-Glu-Ile-Ser-Leu-Met-His-D';

H$_2$N-Ser-Val-Ser-Glu-Ile-Phe-Leu-Met-His-D';

H$_2$N-Ser-Val-Ser-Glu-Ile-Ser-Leu-Met-His-D';

H$_2$N-Ser-Val-Tyr-Glu-Ile-Gln-Leu-Met-His-D';

H$_2$N-Ser-Val-Tyr-Glu-Ile-Ser-Leu-Met-His-D';

25   H$_2$N-Ser-Val-Tyr-Glu-Ile-Phe-Leu-Met-His-D';

H$_2$N-Ser-Val-Phe-Glu-Ile-Phe-Leu-Met-His-D';

H$_2$N-Ser-Val-Tyr-Glu-Ile-Nle-Leu-Met-His-D'; or

H$_2$N-Ser-Val-Phe-Glu-Ile-D-Nal-Leu-Met-His-D';

H$_2$N-Ser-Val-Phe-Glu-Ile-Orn-Leu-Met-His-D';

30

H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Ala-D';

H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Ser-D';

H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Leu-D';

H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Phe-D';

35   H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Tyr-D';

H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Glu-D';

H$_2$N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Lys-D';

H₂N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Gln-D';

H₂N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-Nle-D';

H₂N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-D-Nal-D';

---

5   H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Ala-D';

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Ser-D';

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Leu-D';

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Phe-D';

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Tyr-D';

10   H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Glu-D';

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Lys-D';

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Gln-D';

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Nle-D'; or

H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-D-Nal-D';

15   H₂N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-Orn-D';

H₂N-Ala-Val-Phe-Glu-Ile-Gln-Phe-Met-His-D';

H₂N-Ala-Val-Phe-Glu-Ile-Ser-Phe-Met-His-D';

H₂N-Ala-Val-Ser-Glu-Ile-Phe-Phe-Met-His-D';

20   H₂N-Ala-Val-Ser-Glu-Ile-Ser-Phe-Met-His-D';

H₂N-Ala-Val-Tyr-Glu-Ile-Gln-Phe-Met-His-D';

H₂N-Ala-Val-Tyr-Glu-Ile-Ser-Phe-Met-His-D';

H₂N-Ala-Val-Phe-Glu-Ile-Phe-Phe-Met-His-D';

H₂N-Ala-Val-Tyr-Glu-Ile-Phe-Phe-Met-His-D';

25   H₂N-Ala-Val-Nle-Glu-Ile-Phe-Phe-Met-His-D'; or

H₂N-Ala-Val-D-Nal-Glu-Ile-Phe-Phe-Met-Phe-D'.

or the pharmaceutically acceptable salts thereof,

wherein D' is selected from the 10-34 amino acid
sequence for human or bovine PTH or their Z=-COOH or COO-+M
30   or -(C=O)NH₂ derivatives, or the remaining 10-84 sequence of
pPTH, bPTH, pPTH or hPTHrP in combination with a
pharmaceutically acceptable excipient.

16. The pharmaceutical composition of Claim 15
wherein A', B' and D' are the hPTH(1-34) terminating in -
COOH.

17. The pharmaceutical composition of Claim 15
wherein A', B' and D' are hPTH(1-34) terminating in COO-+M,

wherein M is selected from sodium, potassium, calcium or barium.

18. The pharmaceutical composition of Claim 15 wherein the amino acids at position 1 and 3 are L-serine and A', B' or D' are hPTH(1-34) terminating in Z=-COOH or COO-+M or -(C=O)NH$_2$.

19. The pharmaceutical composition of Claim 15 wherein the amino acid at position 1 is L-alanine, B is serine, A', B', and D' are bPTH(4-34) terminating in Z=-COOH or COO-+M or -(C=O)NH$_2$.

20. The pharmaceutical composition of Claim 15 wherein the amino acid at position 1 is L-serine and the amino acid at position 3 is not L-serine and A', B' or D' are hPTH(1-34) terminating in Z=-COOH or COO-+M or
5   -(C=O)NH$_2$.

21. The pharmaceutical composition of Claim 15 where the amino acid at position 1 is L-alanine and the amino acid at position 3 is not L-serine and A', B' or D' are bPTH(1-34) terminating in Z=-COOH or COO-+M or -(C=O)NH$_2$.

22. The pharmaceutical composition of Claim 15 wherein the amino acid at position 1 is serine, the amino acid position 3 is serine, the amino acid at position 6 is not L-glutamine and A', B' or D' are hPTH(1-34) terminating
5   in Z=-COOH or COO-+M or -(C=O)NH$_2$.

23. The pharmaceutical composition of Claim 15 wherein the amino acids at positions 1 and 3 are L-serine, the amino acid at position 6 is L-glutamine and the amino acid at position 9 is not L-histidine and group D' is
5   hPTH(10-34) terminating in Z=-COOH or COO-+M or -(C=O)NH$_2$.

24. The pharmaceutical composition of Claim 1 wherein Z is (C=O)NH$_2$.

25. The pharmaceutical composition of Claim 15 wherein Z is (C=O)NH$_2$.

26. A pharmaceutical composition comprising a compound of Claim 1 or a pharmaceutically acceptable salt thereof in admixture with a pharmacologically acceptable excipient.

27. A method of treatment of a mammal in need of therapeutic treatment, which method comprises administration of a therapeutically effective amount of the pharmaceutical composition of Claim 1 or the pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable excipient.

28. The method of Claim 27 which comprises administration of a therapeutically effective amount of structure I by oral, parenteral, subcutaneous, intramuscular, intravenous, vaginal, rectal, buccal, sublingual or intranasal means.

29. The method of Claim 28 wherein the compound is used to treat cancer, osteoporosis, hypercalcemia or hyperparathyroid conditions in a human being.

30. A method for selecting polypeptide sequences modified at the 3, 6, 9 position or combinations thereof of a PTH or PTH(1-34) useful in pharmaceutical compositions, which method comprises:

(a) preparing an amino acid sequence of hPTH, bPTH, pPTH, hPTHrP, bPTHrP, hPTH(1-34), bPTH(1-34) or pPTH(1-34) wherein the amino acid at the 3, 6, 9 position or combinations thereof, are replaced by different D- or L- natural amino acids or unnatural amino acids;

(b) performing an assay using specific tissues, membranes, or cells to evaluate receptor binding and activity;

(c) performing an assay using specific bone cells to evaluate receptor binding and activity;

(d) (i) independently selecting those peptide amino acid analogs for further evaluation, having a high binding (B) and high activity (AC) in the specific tissues, membranes or cells and a high specific bone cell binding (B) and high activity (AC), as agonists for medical treatment for disease conditions of the tissues, membranes, cells or bone, or alternatively;

(ii) independently selecting those peptide amino acid analogs for further evaluation, having a high binding

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(B) and high activity (AC) as specific agonists for medical treatment in the specific tissues, membranes, or cells and a low specific bone cell binding (B) and low activity (AC) for disease conditions of the tissues, membranes, or cells,

5   or alternatively;

(iii) independently selecting those peptides for further evaluation as agonists having a high binding (B) and low activity (AC) in specific tissues, membranes or cells and high specific bone cell binding (B) and high

10   activity (AC) for disease conditions of the bone, or alternatively;

(iv)   independently selecting high binding (B) and low activity (AC*) in tissues, membranes or cells and in bone cells for use as antagonists in the medical

15   treatment of hormonal disorders and cancers; and

(e) performing subsequent different assays or toxicity determinations on the amino acid analogs pursuant to identifying a useful pharmaceutical.

31.   The method of Claim 30 wherein

in step (d) (i) the high binding (B) and high activity (AC) in the specific tissues, membranes or cells and a high specific bone cell binding (B) and high activity (AC) is

5   about 50% or greater compared to that of the reference peptide.

32.   The method of Claim 30 wherein

in step (d) (ii) the high binding (B) and high activity (AC) as agonists for medical treatment in the specific soft tissues, membranes, or cells is about 50% or

5   greater compared to that of the reference peptide and the low specific bone cell binding (B) is about 0% and low activity (AC) of bone cell is about 10% or less.

33.   The method of Claim 30 wherein

in step (d) (iii) the low binding (B) is less than 10% of the reference and the low activity (AC) is about 0% in specific soft tissue, and high specific bone cell binding

5   (B) and the high activity (AC) is 50% of the reference or greater, for disease conditions of the bone.

34. The method of Claim 30 wherein

in step (d) (iv) the high binding (B) is greater than 50% and the low activity (AC*) is about 0% in tissues, membranes or cells or in bone, for use as antagonists in

5   the medical treatment of hormonal disorders and cancers.

35. The method of Claim 30 wherein the soft tissues, membranes or cells are derived from kidney.

	1				5					10					15					20					25					30				
PTH bovine	A	V	S	E	I	Q	F	M	H	N	L	G	K	H	L	S	S	M	E	R	V	E	W	L	R	K	K	L	Q	D	V	H	N	F
PTH human	S	V	S	E	I	Q	L	M	H	N	L	G	K	H	L	N	S	M	E	R	V	E	W	L	R	K	K	L	Q	D	V	H	N	F
PTH rat	A	V	S	E	I	Q	L	M	H	N	L	G	K	H	L	A	S	V	E	R	M	Q	W	L	R	K	K	L	Q	D	V	H	N	F
PTH chicken	S	V	S	E	M	Q	L	M	H	N	L	G	E	H	R	H	T	V	E	R	Q	D	W	L	Q	M	K	L	Q	D	V	H	S	A
PTH porcine	S	V	S	E	I	Q	L	M	H	N	L	G	K	H	L	S	S	L	E	R	V	E	W	L	R	K	K	L	Q	D	V	H	N	F
PTHrP human	A	V	S	E	H	Q	L	L	H	D	K	G	K	S	I	Q	D	L	R	R	R	F	F	L	H	H	L	I	A	E	I	H	T	A
PTHrP chicken	A	V	S	E	H	Q	L	L	H	D	K	G	K	S	I	Q	D	L	R	R	I	F	L	Q	N	L	I	E	G	V	N	T	A

FIG._1
(PRIOR ART)
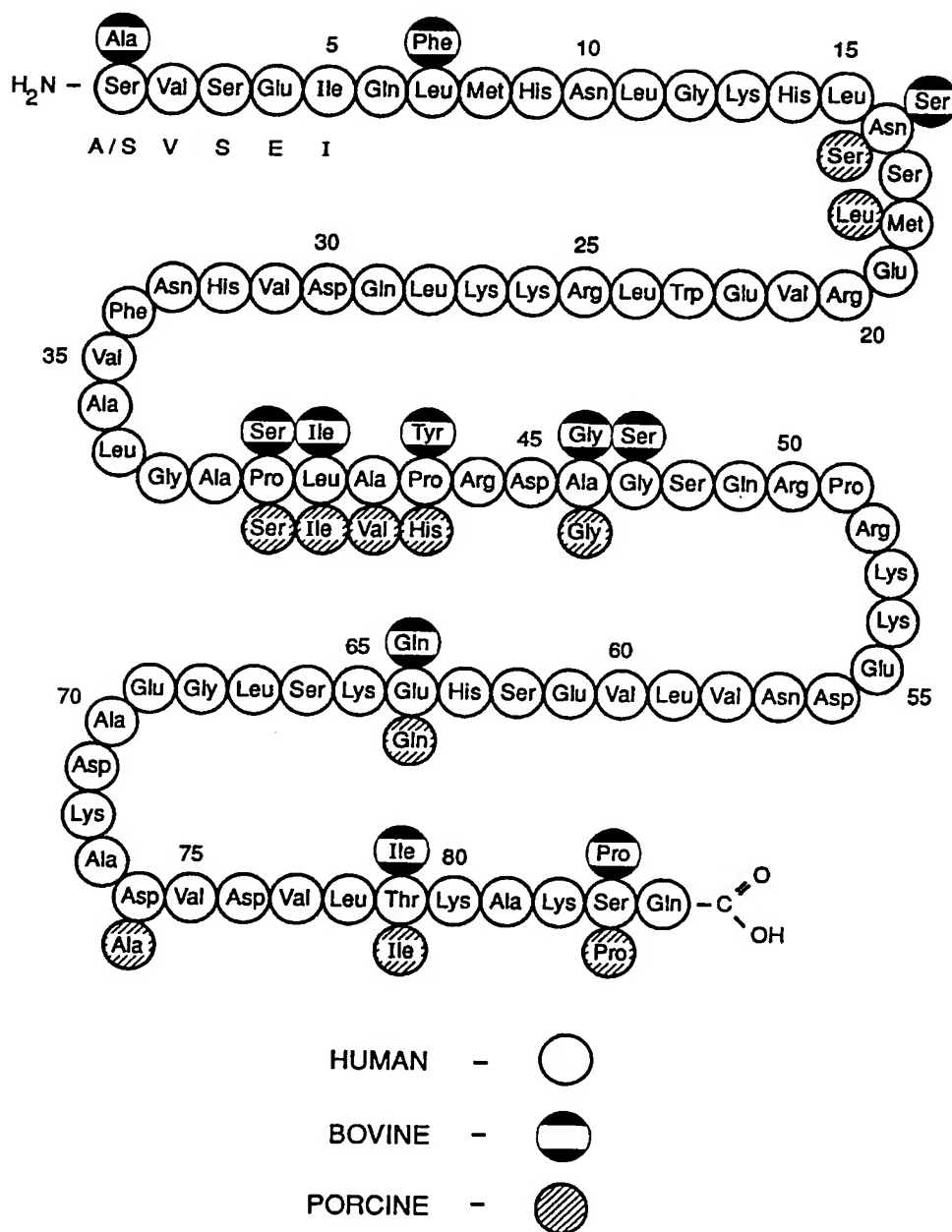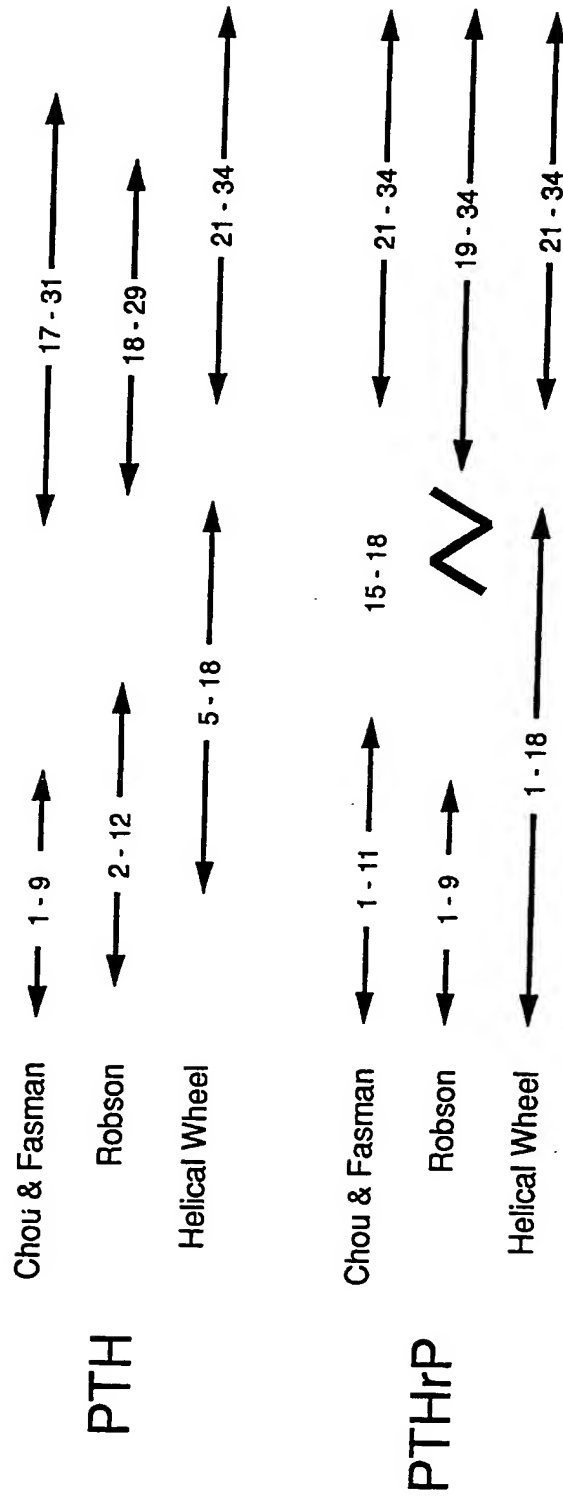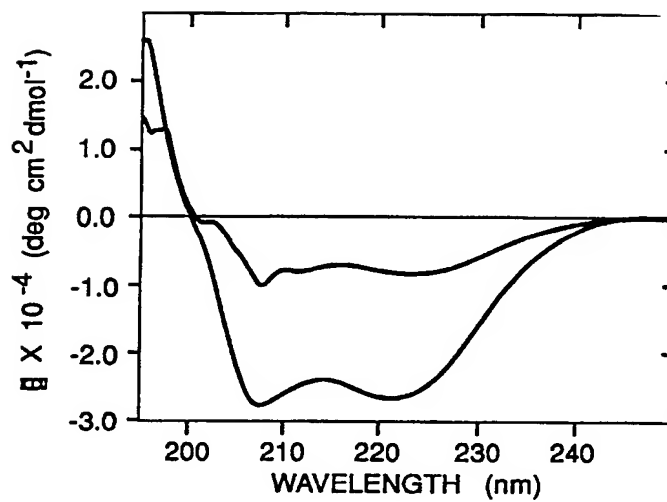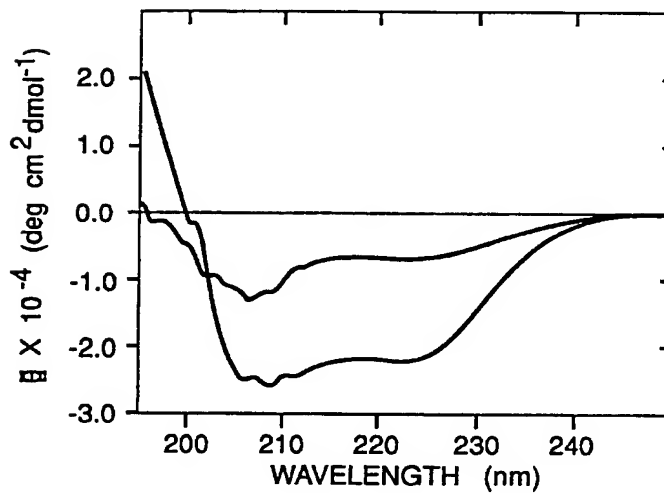
FIG._1A

(PRIOR ART)

FIG._2
(PRIOR ART)

FIG._3A

FIG._3B

FIG._3C
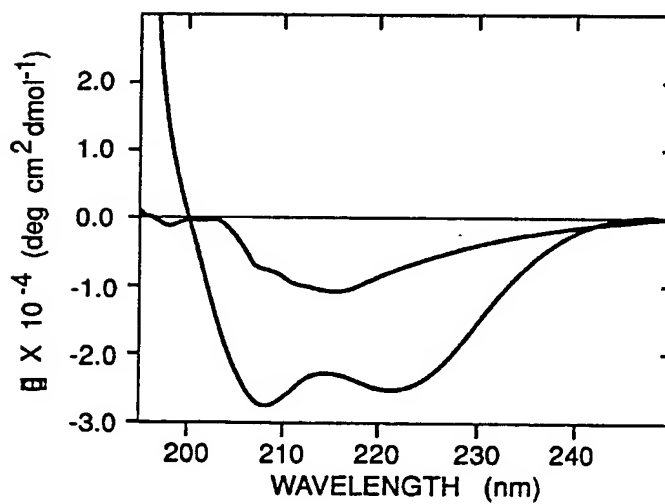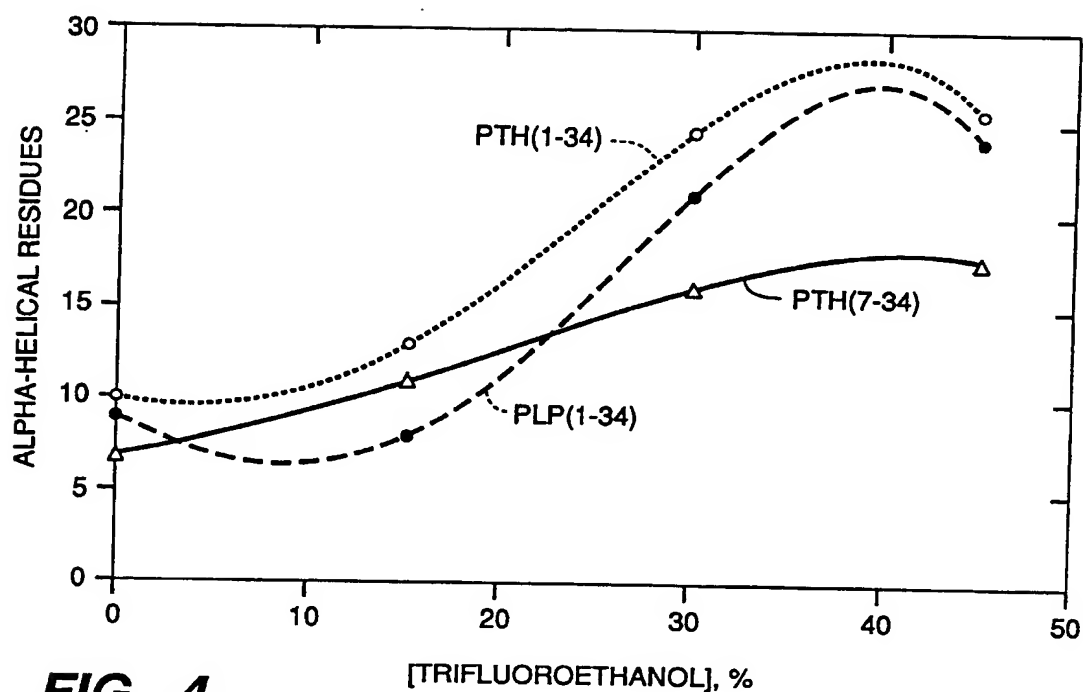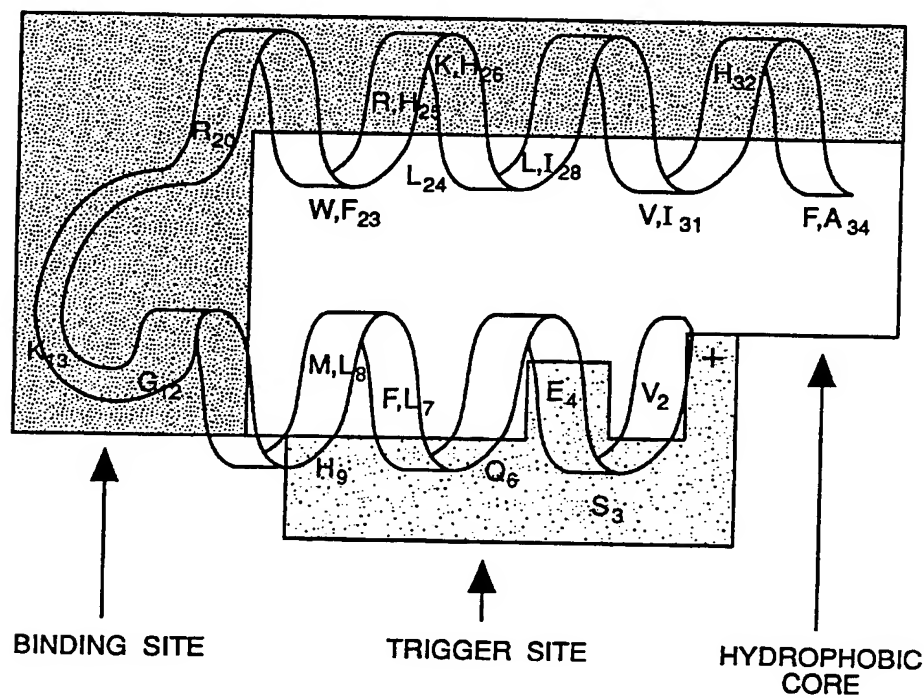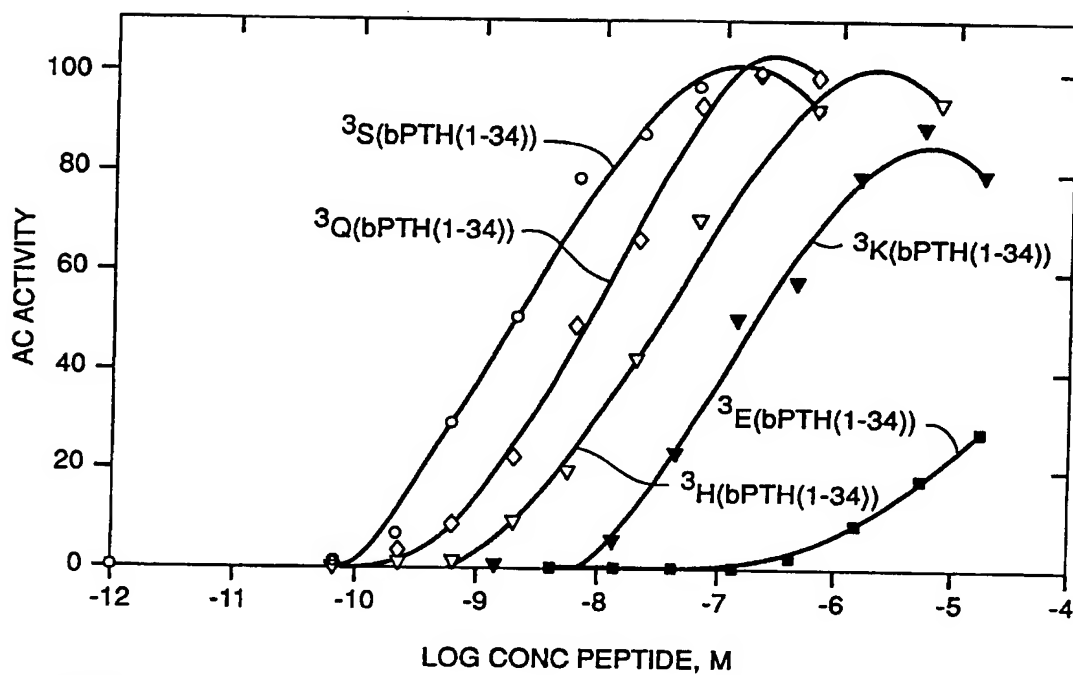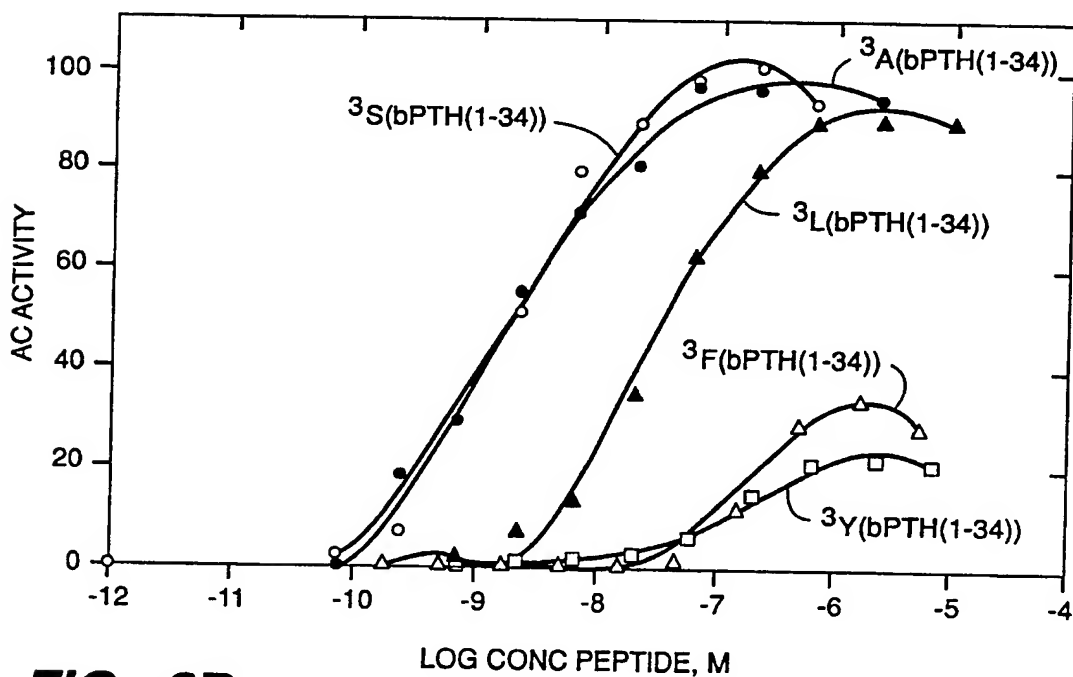
FIG._4

BINDING SITE   TRIGGER SITE   HYDROPHOBIC CORE

FIG._5

FIG._6A

FIG._6B
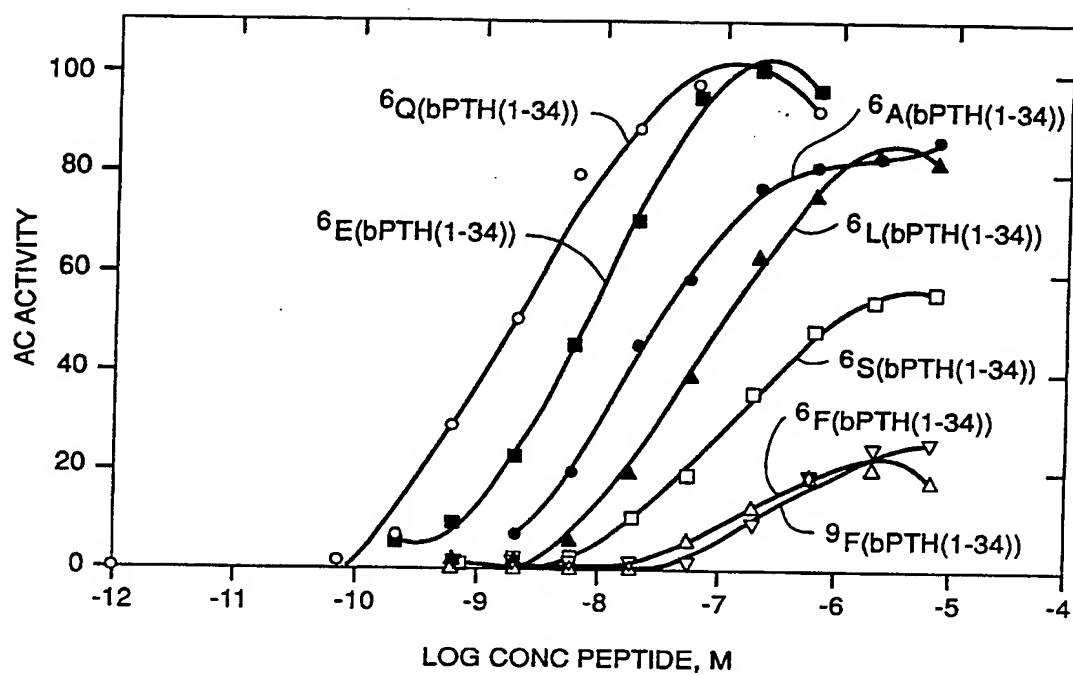
FIG._6C
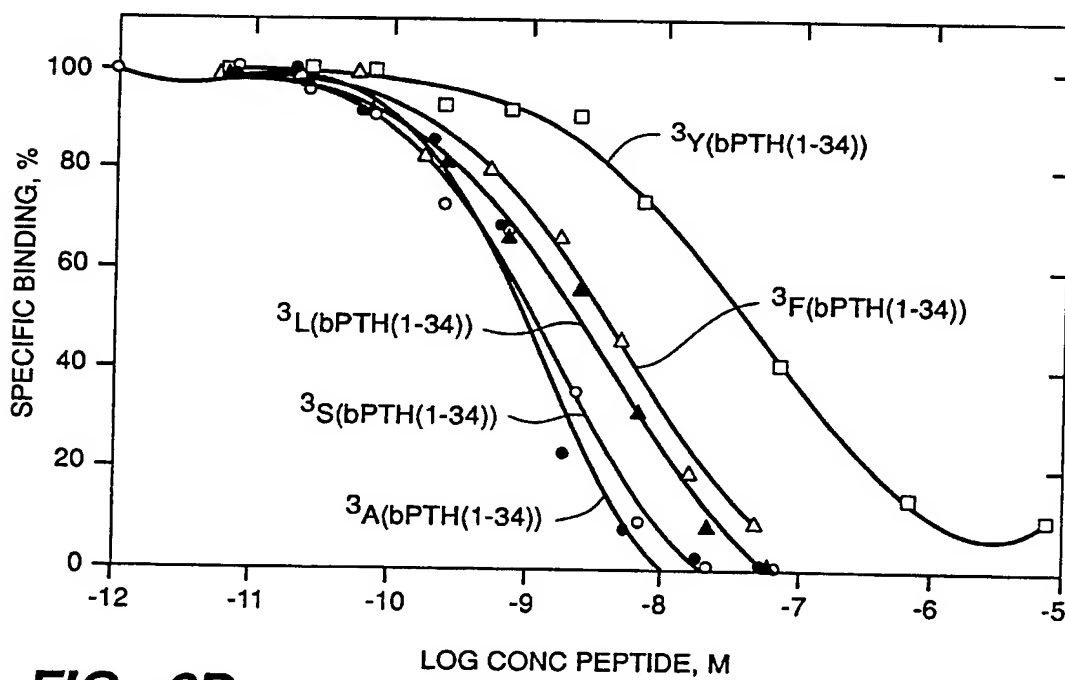
FIG._6D
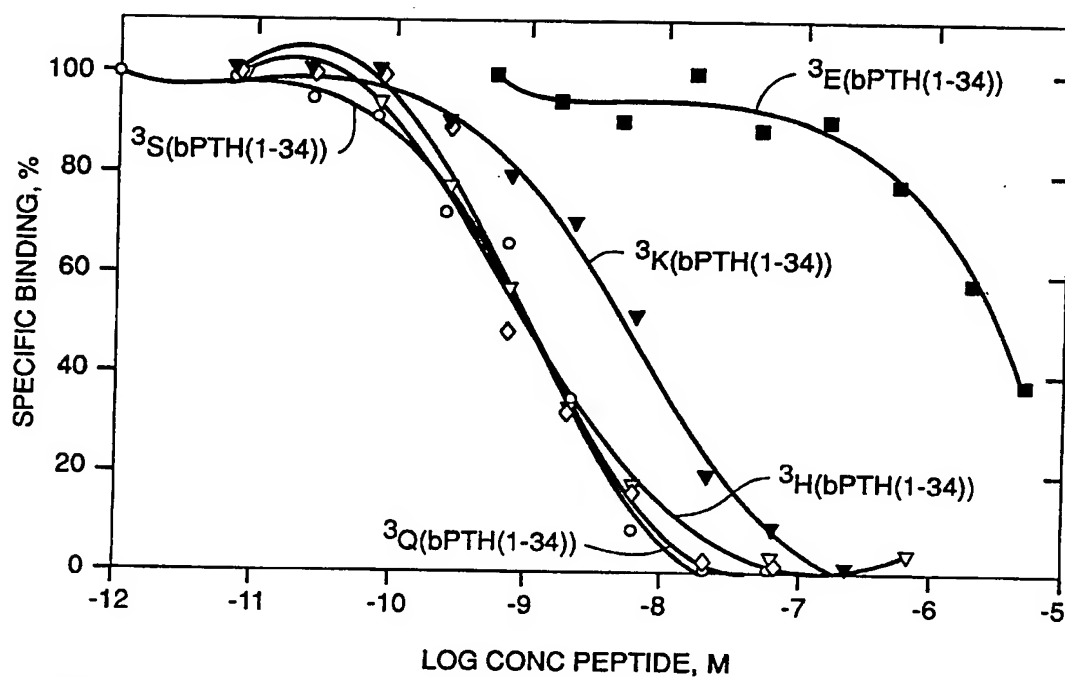
FIG._6E
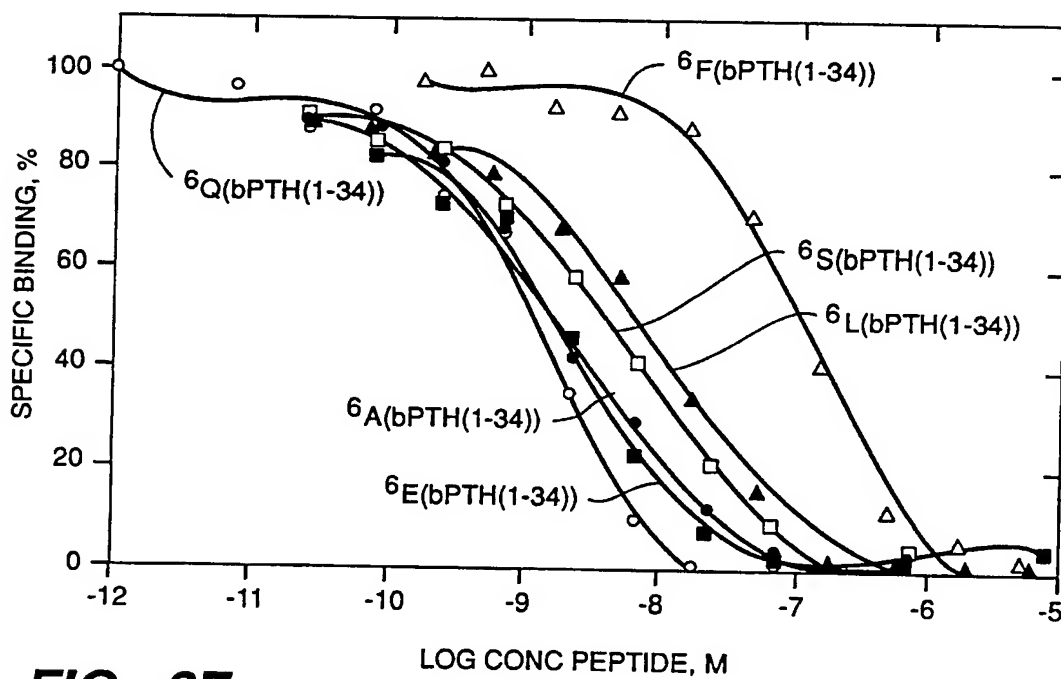
FIG._6F
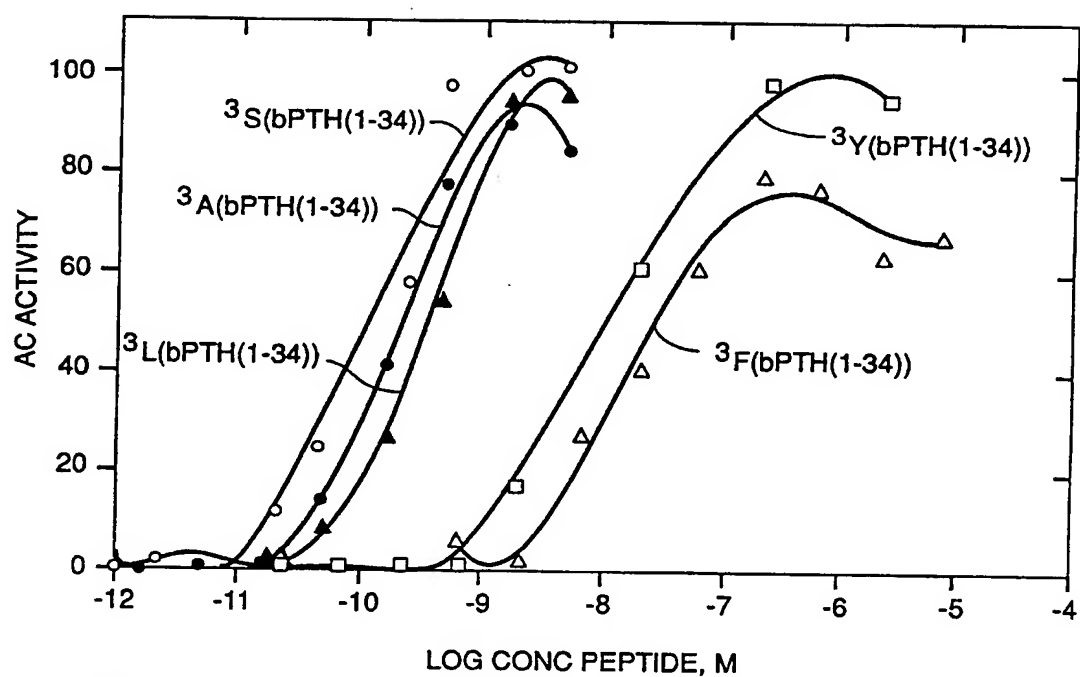
FIG._7A
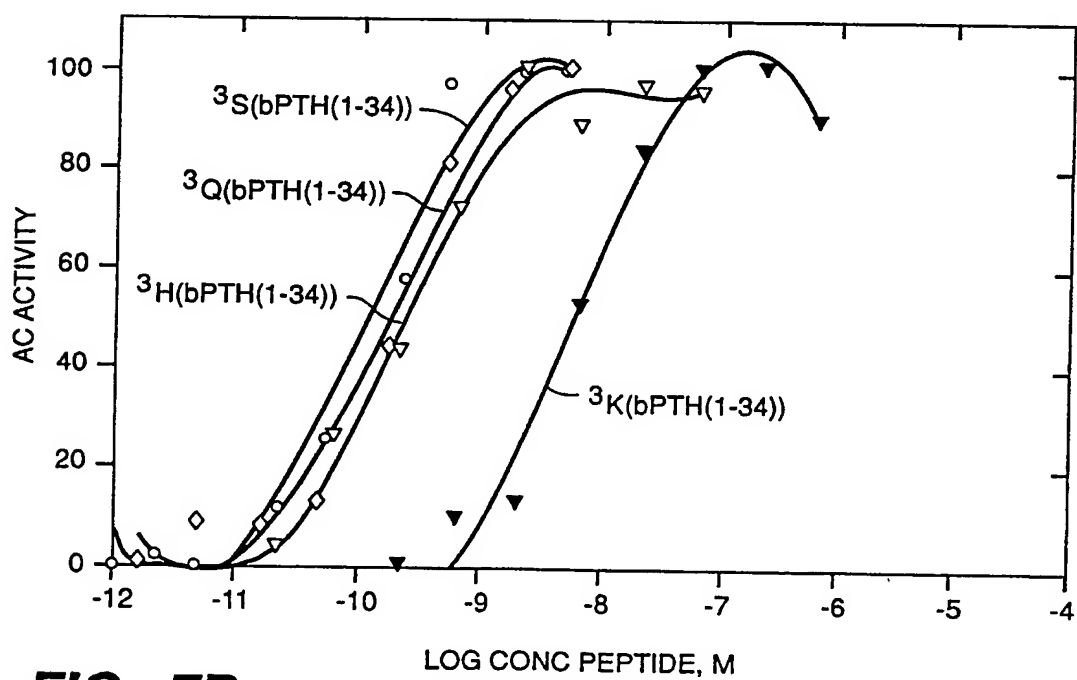
FIG._7B
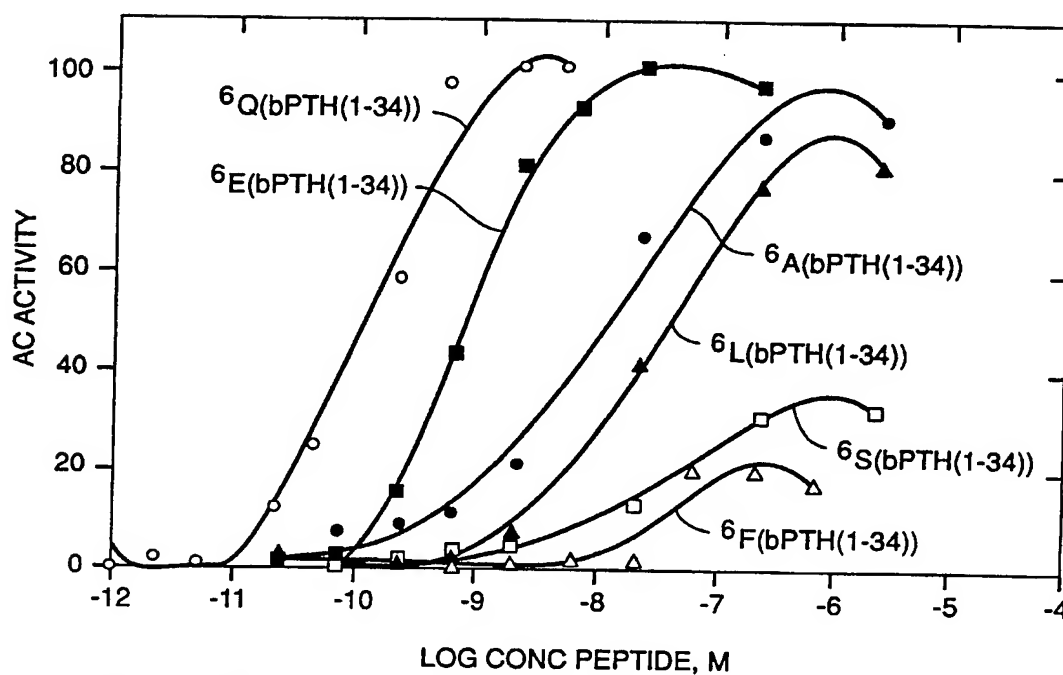
FIG._7C
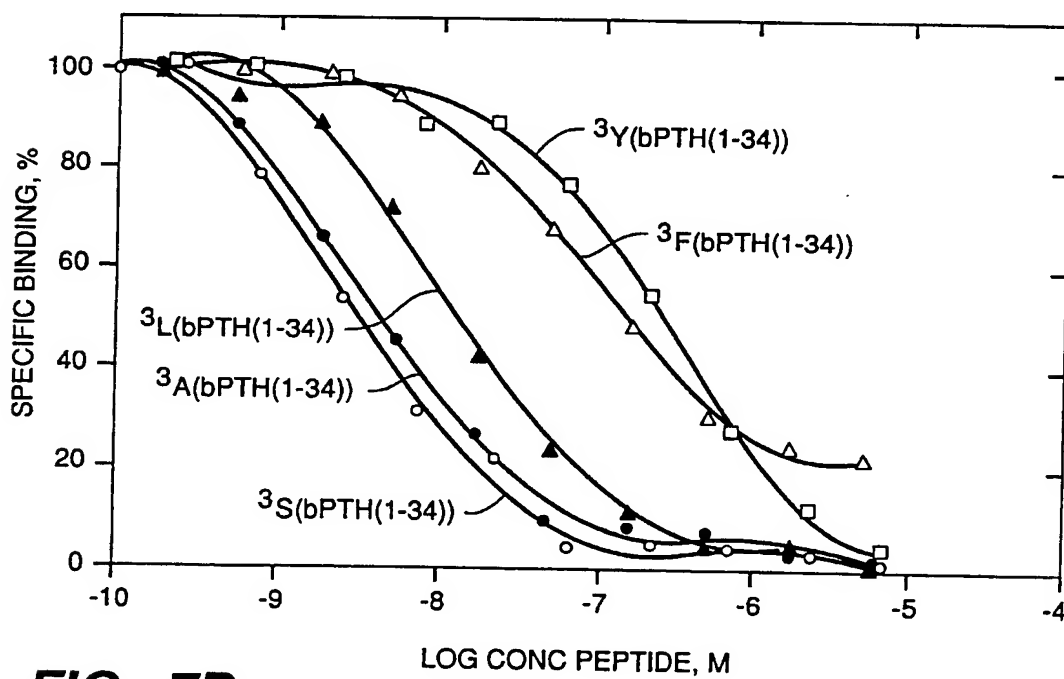
FIG._7D
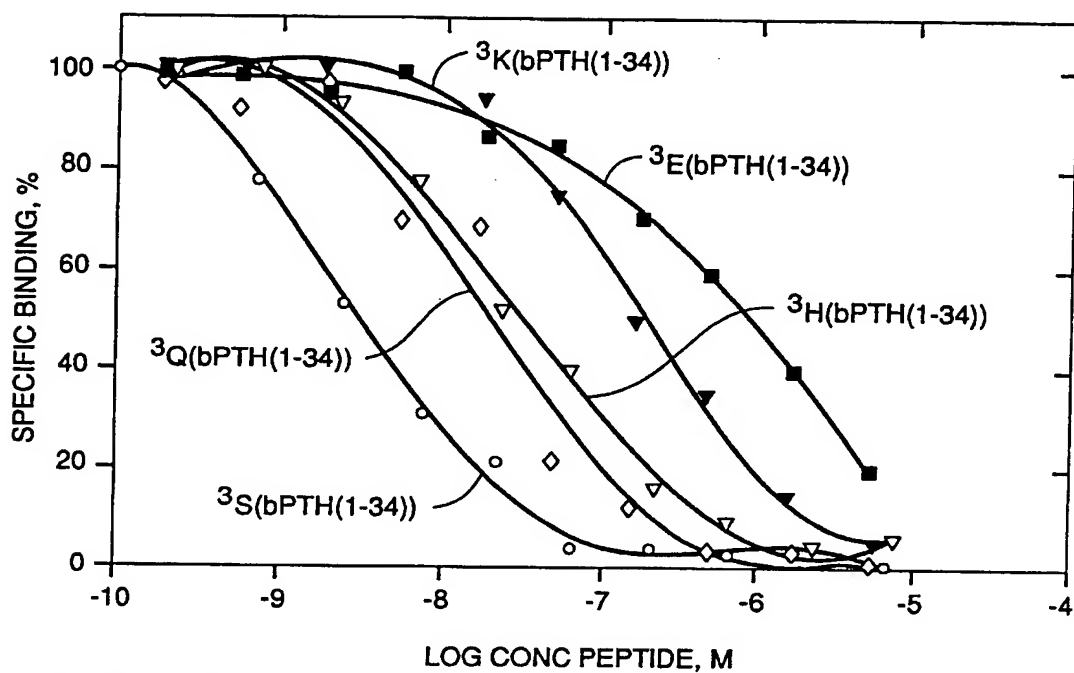
FIG._7E
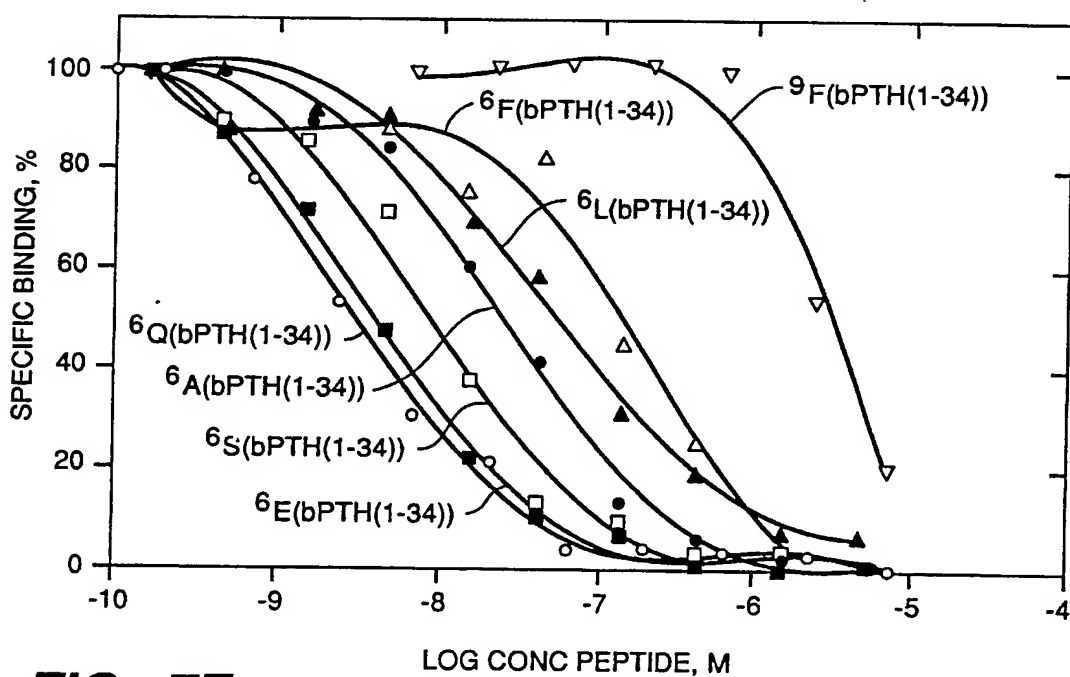
FIG._7F
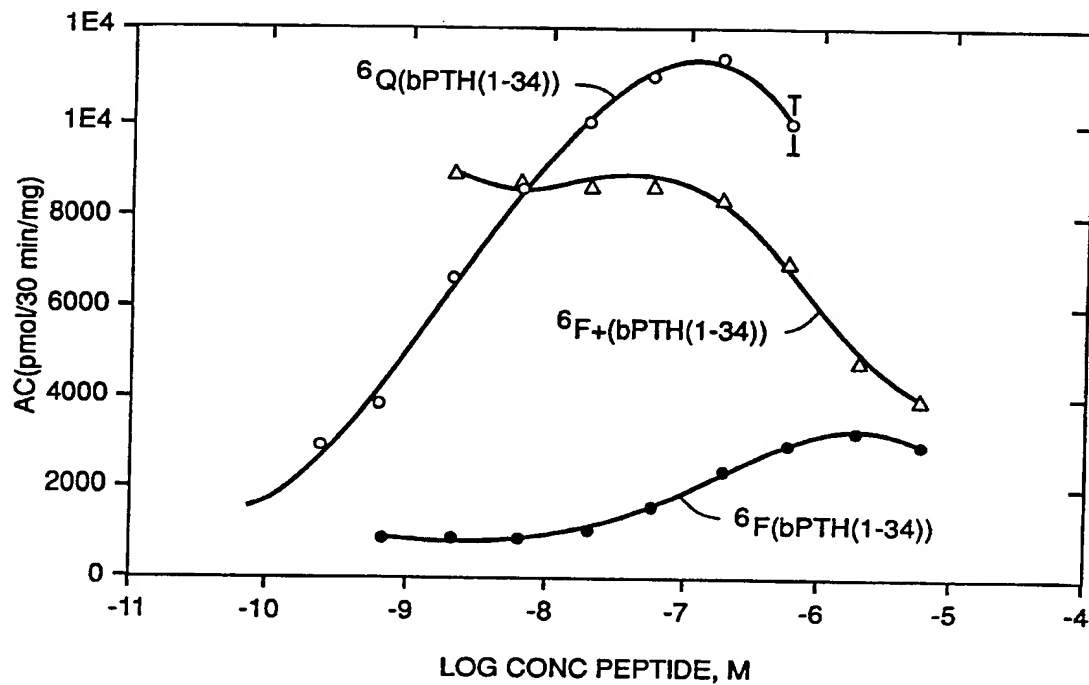
FIG._8A
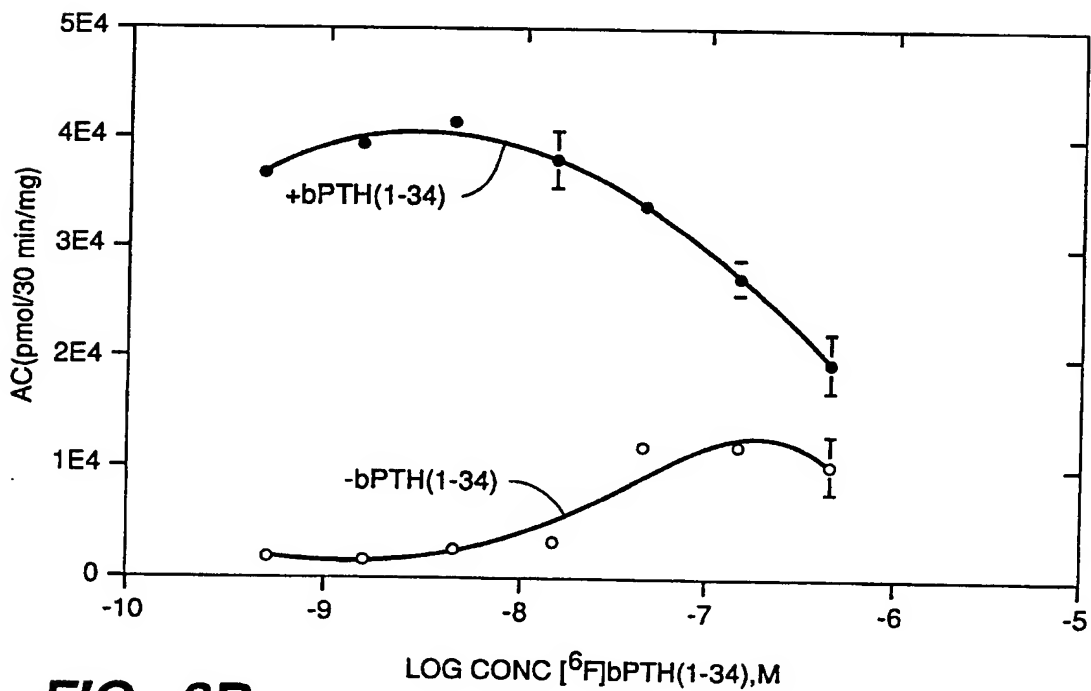
FIG._8B

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/30; C07K 3/04, 7/36; G01N 33/15, 33/74, 33/566
U.S. Cl.: 530/324; 514/12; 436/86, 501

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
U.S. Cl.	514/12; 530/324; 436/86, 501

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched 8

APS, search terms: parathyroid (w) hormone?, agonist?, antagonist?, assay?, bone(w) cell?

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X,P	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 266, No.3, issued 25 January 1991, F.E. Cohen et al., "Analogues of Parathyroid Hormone Modified at Positions 3 and 6", pages 1997-2004, see entire document.	1-35
Y	US,A, 4,508,828 (LINDAILET AL) 02 April 1985, see column 5, line 50 to column 6, line 30, and column 1, line 65 to column 2, line 68.	30-35
Y	ACTA ENDOCRINOLOGICA, Volume 100, No.3, issued July 1982, R. Zonefrati et al.: "Parathyroid hormone bioassay using human kidney cortical cells in primary culture" pages 398-405, see Abstract.	30-35

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

08 OCTOBER 1991

Date of Mailing of this International Search Report

07 NOV 1991

International Searching Authority

ISA/US

Signature of Authorized Officer

STEPHEN WALSH

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET		
Y,P	US,A, 4,968,669 (ROSENBLATT et al.) 06 November 1990, see columns 7 and 8.	30-35
A	US,A, 4,771,124 (ROSENBLATT et al.) 13 September 1988.	15-21
Y	US,A, 3,886,132 (BREWER et al.) 27 May 1975, see entire document.	15,21
Y	G. SCHULZ et al., "PRINCIPLES OF PROTEIN STRUCTURE", published 1979 by Springer-Verlag (N.Y.), see pages 14-16.	15,21

V.☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.☐ Claim numbers . , because they relate to subject matter ¹³ not required to be searched by this Authority, namely:

2.☐ Claim numbers .... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

3.☐ Claim numbers_____ , because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI.☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

See attached

1.☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4.☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2) (Rev. 11-87)

Attachment to Form PCT/ISA/210/Part VI

Observations where unity of invention is lacking

Detailed reasons for holding lack of unity of invention

The claims of the two groups are directed to distinct methods having the characteristics of two distinct inventive concepts. The method of group I pertains to the administration of a pharmaceutical composition to a subject. The method of group II pertains to an in vitro assay method. PCT Rule 13.2 does not provide for distinct methods within the same inventive concept.

Itemized summary of claims groupings

I. Claims 1-29, drawn to pharamaceutical compositions and method of administering the compositions, classified in class 514, subclass 12.

II. Claims 30-35, drawn to methods for selecting useful polypeptide sequences, classified in class 536, subclass 86.